INSIGHTS IN RHEUMATOLOGY: 2021

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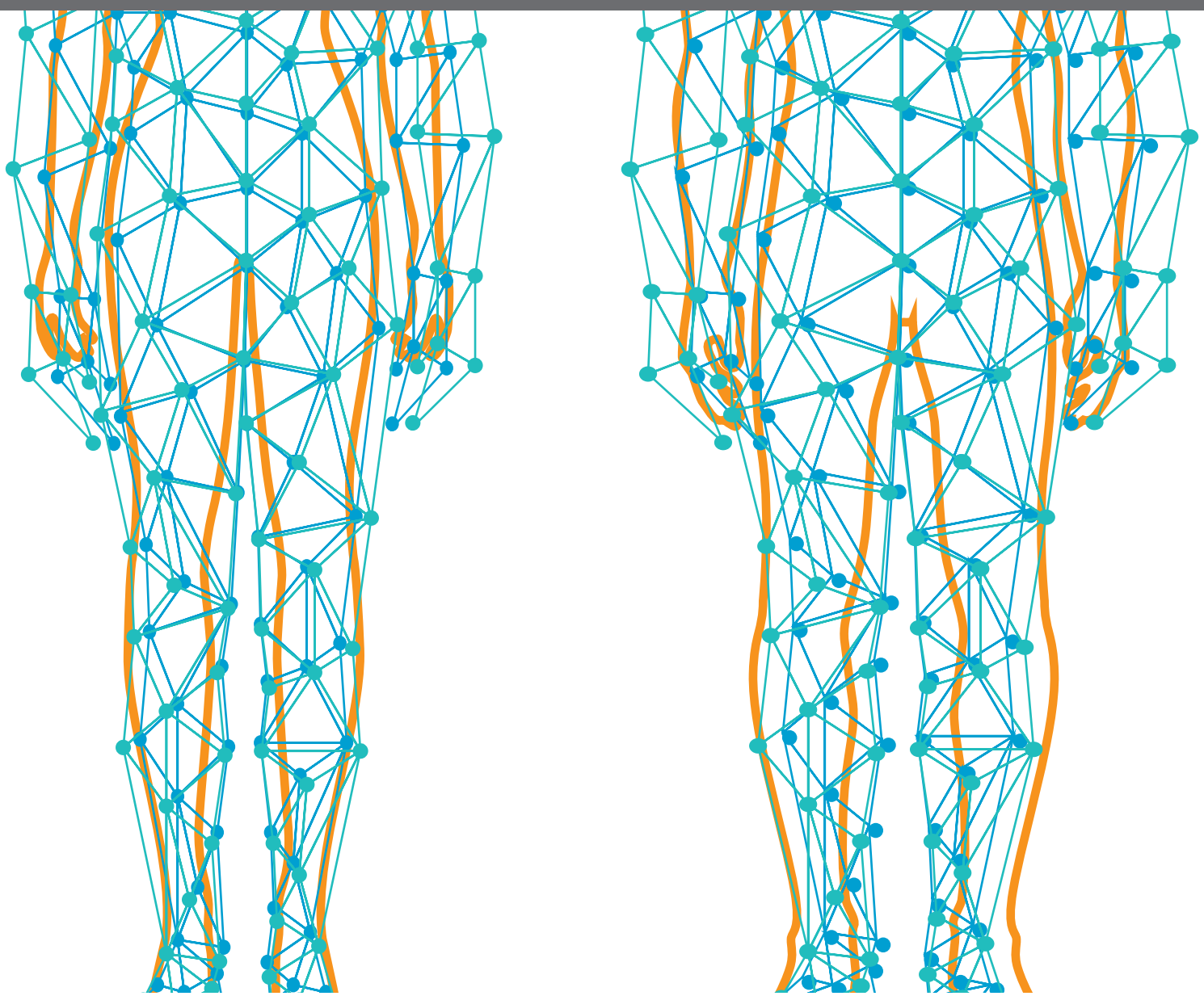

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Editorial: Insights in Rheumatology: 2021

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IL-33, B cells, synovial fibroblasts, regenerative medicine, pre RA

Editorial on the Research Topic
Insights in Rheumatology: 2021

In this Research Topic, entitled *Insights in Rheumatology: 2021*, a broad range of subjects were highlighted, from fibromyalgia to gout, also encompassing novelties on the role of Interleukin-33 (IL-33) and new treatment paradigms such as regenerative medicine, giving a glimpse of the heterogeneity of rheumatic diseases.

In a very interesting and stimulating manuscript, Maugars et al. highlighted the concept of hypersensitization as a way of integrating a complex symptom constellation that goes beyond generalized pain in the context of fibromyalgia. The authors also discuss the role of rheumatologists in the approach to this condition, emphasizing the relevance of the differential diagnosis. Other concepts such as coping, resilience, catastrophizing, and the possible relationship with post-traumatic syndromes underline the psychologic dimension of the fibromyalgia patient. Furthermore, neuro-imaging findings have allowed a better understanding of the pathology of fibromyalgia as a disorder of the cortical integration of chronic pain, with amplification of sensory nociception signals and a decrease in pain perception threshold, combined with a persistence of the stimulus that contributes to chronicity (Maugars et al.). Gout is another very frequent challenge facing rheumatologists and it is arguably one of the "oldest" rheumatic diseases. However, we still face patients where available treatment options fail to completely control the disease. Thus, it is clearly of interest to understand the clinical experience of a gout Chinese cohort treated with benzbromarone (a nonpurine xanthine oxidase inhibitor), a first-line urate-lowering drug in Asia (Xue et al.).

Rheumatology is still seeking the full understanding of the cytokine networks that are behind chronic immune mediated inflammatory diseases (IMID). IL-33 is a very interesting cytokine in this context. It can be expressed in inflammatory cells and play an immunomodulatory role, activating different cells and inducing cytokine production and thus inflammation through a pathway initiated by the IL-33/serum stimulation-2 (ST2) axis (Dong et al.). This axis has been shown to play a role in the pathophysiology of several rheumatic diseases, including gout, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis (SSc) (Dong et al. and Versace et al.). Of interest, in the context of SSc, levels of IL-33 correlate with skin fibrosis, and this cytokine seems to induce both cutaneous and pulmonary

fibrosis *via* increased IL-13. Versace et al. have found that IL-13 and IL-33 levels were increased in SSc patients and seem to associate with measures of pulmonary disfunction. These observations further reinforce the role of IL-33 in rheumatic diseases and open the possibility of exploring it as a therapeutic target or as a biomarker.

The autoimmune range of the rheumatic diseases' physiopathology spectrum is closely linked to the biology of B cells. From a pragmatic view the effectiveness of biologic therapies that target B cells in these diseases proves the direct or indirect role of these cells in the diseases mechanisms of conditions such as SLE and RA (1). The use of Belimumab in SLE treatment and active lupus nephritis is an example. Parodis et al. have demonstrated that, conflicting with previous findings (2–4), belimumab can induce rapid and sustained decreases in some plasma cell subsets, which does not seem to relate to response to treatment. On the other hand, the expanding-returning pattern observed in memory B cells induced by belimumab seems to be evident in clinical responders. Additionally, authors have demonstrated that clinical response to belimumab was associated with preceding reductions of anti-dsDNA and increases in C3 and C4 levels. The role of B cells is still unclear in the context of juvenile idiopathic arthritis (JIA). However, in subsets of patients, the B cell depleting therapy rituximab can be effective (Moura and Fonseca). Yet a better understanding of the possible common physiopathology mechanisms between RA and polyarticular and extended oligoarticular is needed to further dissect the heterogeneity of JIA.

Synovial fibroblasts are classic players in RA and their role in perpetuating inflammation and leading ultimately to damage is well-known. Thus, targeting these cells, could be and additional approach to achieve and maintain remission in RA (5). Interestingly, these treatment strategy could be particularly beneficial for patients with pauci-immune synovial pathotypes with a poor response to standard therapy (Chu). Current treatment options are not able to specifically interfere with synovial fibroblasts. However, indirectly they can influence the behavior of these cells, as is the example of peficitinib (a JAK inhibitor) that seems to suppress synovial fibroblast migration *in vitro* and may induce fibroblast apoptosis (Chu). Another persistent topic of discussion in RA is the concept of flare. A role of preinflammatory mesenchymal (PRIME) cells has been suggested, as they could actively migrate into the joint and stimulate local inflammation, with a potential contribution of long-term persistence of synovial resident memory T cells in the joint to their homing (Bozzalla-Cassione).

While the role of disease activity indexes is crucial for defining a flare, "patient reported outcomes" have become increasingly relevant, creating the concepts of patient-based and physician-reported flare. On the other extreme of natural history of RA are the subtle initial symptoms that may signalize the very early stage of RA, or even a stage that corresponds to an immune disturbance (for example the presence of anti-citrullinated protein antibodies) that represents a risk for RA, still without an inevitable physiopathology and clinical fate. Despite well-defined classification criteria for RA that allow identification of patients even at early stages of the disease, and thus treat them and prevent disease progression and damage, no definite definitions and clinical approach to patients at-risk for RA are established. Risk is represented by an interaction between environmental, genetic, and immune factors, associated or not with symptoms such as unspecific arthralgias (6). Identification of these patients through a European Registry of at-risk people can prove invaluable to research aimed at preventing progression (Studenic et al.).

While rheumatic diseases have effective treatments available, and the concept of prevention of disease in at-risk individuals seem to be becoming relevant, irreversible damage can be and is often a reality. Yoshimi and Nakajima recall the potential role of regenerative medicine as a paradigm shift in treating rheumatic patients with poor prognosis. Recent research has focused on autologous Hematopoietic Stem Cell Transplantation, and EULAR recommends it for skin and lung disease in rapidly progressive SSc at risk of organ failure (Yoshimi and Nakajima). Mesenchymal stem cell transplantation has been tried in SSc patients with improvement in skin sclerosis and ulcers, and in SLE patients refractory to standard therapy and lupus nephritis (Yoshimi and Nakajima). However, there is not sufficient evidence to formally recommend it in the clinical setting. More research is also for sure needed in the field of induced pluripotent stem cells and vascular endothelial progenitor cells.

Insights in Rheumatology 2021 bring us the richness of the progress in this field but also the unknown shadows that constitute the research agenda for the future.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

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Fibromyalgia and Associated Disorders: From Pain to Chronic Suffering, From Subjective Hypersensitivity to Hypersensitivity Syndrome

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The concept of fibromyalgia has progressed to achieve a certain consensus regarding the definition of the condition. We summarize what is known in 2020, be it in terms of diagnosis, with the criteria that have changed over the years, or at the level of the psychological profile, via the notions of "catastrophizing" and "coping" and post-traumatic syndrome. The importance of fatigue and sleep disorders is underlined, with the chronological sequence of post-traumatic syndrome, chronic fatigue, and then amplification of the pain and the onset of multiple associated symptoms. The etiopathogenic debate has been enriched thanks to neuro-imaging data to discover the start of the central neurological signature. The many associated symptoms are reanalyzed in the context of so-called sister conditions which form sometimes more or less separate entities, such as chronic fatigue syndrome or restless legs syndrome for example. What these conditions have in common is hypersensitivity, not just to pain, but also to all exteroceptive stimuli, from deep sensitivity in the neuro-vegetative system, the sense organs and certain functions of the central nervous system, to the psychological aspects and sleep control. In summary, it is possible to define fibromyalgia as a cognitive disorder of cortical integration of chronic pain, with amplification of painful and sensory nociception, decrease in the threshold for the perception of pain, and persistence of a stimulus that maintains the process in chronicity. Fibromyalgia is part of a group of chronic hypersensitivity syndromes of central origin, with a very wide range of means of expression.

Keywords: fibromyalgia, pain, fatigue, diagnosis, psyche, neuro-imaging, hypersensitisation

INTRODUCTION

Fibromyalgia is a very common condition. Its concept was discussed for many years before being as well-defined as it is now. The first false idea was to believe that it was a new condition. Back in the nineteenth century, there were already descriptions that were perfectly typical of fibromyalgia, for example by Beard (1). Then, for most of the twentieth century, the condition was called fibrositis

(2). The many subsequent studies led to the statement that there was indeed pain, but on the contrary no sign of inflammation, meaning that the suffix -itis was not appropriate. This was why Hench created the term "fibromyalgia," this time using the suffix -algia. The term has been used for around 40 years now, in line with the development around the world of anti-pain centers (3). Yunus took up the term in the 1980s, and it remains in use today even though, as we shall see, pain is only one of the components in a complex set of symptoms (4). We will thus use the term fibromyalgia throughout this review. As we shall see, another concept has emerged, led by Yunus, that of neurosensory amplification: hypersensitization.

The typical fibromyalgia profile can be described in just a few words. The patient is a young woman who has suffered from pain, in an intense manner, all over, for several months or years and without any diagnosis being made. More than 9 times out of 10, patients are women, although there is no clear explanation for this. They are more often than not young, in the 20–60-year range. After the age of 60, fibromyalgia is less frequently diagnosed. The prevalence in Minnesota showed a trend of a decrease with the age, respectively, 8.45%/6.02%/3.79% in 21–39/40–59 and over 60+ years groups (5). But only there were only 27.6% responders to a survey which was sent by mail, which is less used by older people. Il is possible that over 60 years old generalized pain, fatigue and sleep disorders could be more easily attributed to osteoarthritis and old age. Before the age of 20, fibromyalgia is also rare, but we are nevertheless seeing fibromyalgia patients who are increasingly young, with a prevalence that remains constant (6). The costs of fibromyalgia are widely underestimated. A study carried out in the United States compared over 1 year all the costs associated with fibromyalgia with rheumatoid arthritis (7). Globally speaking, there was little difference: $12,000 for fibromyalgia and $14,700 for rheumatoid arthritis. In more detail, although rheumatoid arthritis is associated with high treatment costs, because of biotherapies, we can see that the difference is not as much as all that. On the other hand, fibromyalgia is more costly in other areas, such as imaging, medical biology, and physiotherapy. Although rheumatoid arthritis is a condition with potential joint destruction and deformation, which is not the case in fibromyalgia, there are significantly more days off work for fibromyalgia, with an equivalent degree of invalidity. Fibromyalgia thus has a high annual cost because of its medical nomadism, the requests for multiple and often unnecessary examinations, and finally in the search for a wide range of treatments which are added on despite their poor efficacy. Furthermore, fibromyalgia is 5–10 times more common than rheumatoid arthritis.

We will approach the condition first from a classic point of view, passing from the concept of pain to that of suffering, involving complex psychological integration phenomena. We will then discuss the psychological characteristics, the etiopathogenic aspects and the major input of neuro-imaging. Finally, we will discuss the modern concept of hypersensitization syndrome of central origin.

TYPICAL SYMPTOMS

The symptomology, like the reason for consulting a doctor, is dominated by pain. It has an essentially lumbar and gluteal, cervical and scapular, dorsal paravertebral axial topography. It predominates above all at the level of the cervico-scapular and lumbar-pelvic belts. A study has compared to a painless group the intensity and location of pain in 5 painful conditions: painful temporomandibular disorder, headache, low back pain, irritable bowel syndrome, and fibromyalgia (8). Fibromyalgia is clearly individualized by whole posterior axial pain and headache (temple, behind eyes, forehead, and inside), and with less pain at feet, arms and thighs. So peripheral pain in the limbs is rarer, although it has been observed in the knees, elbows and hands. The intensity of the pain is significant, often between 6 and 10, with a non-negligible proportion of patients who would put their pain at more than 10 on the pain scale, even though they maintain their regular daily activities (9). Mean evaluation of the pain in fibromyalgia is much more important than for rheumatoid arthritis or lupus, respectively, at 6,0–4,1 and 3,9 in a comparative study (10). The pain can be present for many months, often several years or even decades.

Fibromyalgia pain varies significantly over time, depending on the period, the season, and the activities (11). Certain factors thus aggravate the pain, the most important of which is inactivity (12). A vicious circle then develops: the pain forces patients to stop certain activities, and the consequence of this is even more pain. On the contrary, sport, which is, along with physical activity, an element of choice in the treatment, together with heat and relaxation, are factors that are favorable and on which it is necessary to count. Humidity and cold also have an impact on the pain, as is the case for rheumatic pain (13). Stress is a very clear aggravating factor (14). Alcohol could be a factor that improves the pain, although obviously it is not therapeutics (15). Finally, fatigue is clearly associated with aggravation of the pain, as we shall see.

Fibromyalgia is initially difficult to diagnose because the associated signs can simulate many other conditions (16). Here we can mention for example paresthesia of the upper or lower limbs, which can suggest carpal tunnel syndrome or any other entrapment syndrome or polyneuritis; an acrosyndrome can suggest Raynaud's syndrome; mouth dryness can simulate Gougerot-Sjögren syndrome; feelings of swelling can simulate rheumatoid arthritis or spondyloarthritis or psoriatic arthritis; morning stiffness can simulate polymyalgia rheumatica; headaches can resemble migraines; balance disorders can resemble vertigo; and finally, atypical digestive, urinary, visual and auditory disorders have been reported.

It is also very important to screen for the signs associated with the pain, as they can have both a diagnostic and prognostic value. Sleep disorders are almost constant, of long date, with insomnia or sometimes sleep that feels real but which is not in fact restorative (17). This is how the fatigue takes hold, from the moment the patient wakes up in the morning. The intensity, which is often considerable, confines patients to inactivity and is described as a feeling of total exhaustion. It should be noted that sleep apnea syndrome is associated with fibromyalgia in almost

one in three cases, and this should be remembered systematically in obese female patients who snore (18). There can also be muscle fatigue. Other important associated signs should also be screened for, even if they are not reported spontaneously by the patient. These include headaches, in approximately one case in two, anxiety, stress and depression in approximately one case in three, and irritable bowel syndrome, also in one case in three (post).

In contrast with the clinical picture of very intense pain with a wide variety of so-called functional or subjective associated signs noted by the patients, the objective examination is perfectly normal (8). There is no synovitis, no tenosynovitis, which can be verified by ultrasound or MRI, no tendinitis, even though the pain points are more often than not located in the same enthesopathic areas. There is also no objective alteration to muscle strength, even though myalgia can be found on deep palpation, and the neurological examination is normal. There are no significant or specific cutaneous signs either. What can be found on clinical examination are multiple pain points: diffuse, bilateral, and symmetrical, the core of the old ACR diagnostic criteria for fibromyalgia, dating from 1990 (19). They correspond to spontaneous reported pain, above all axial in two belts: the cervical-scapular belt and the lumbopelvic belt, but also at the anterior thoracic level. There are fewer peripheral pain points and the most characteristic are the lateral epicondyles, crow's feet, trochanters, the greater tuberosity of the humerus and the internal supramalleolar muscles. It is possible to use sensors to visualize the allodynia where the pressure is low but where a simple handshake or discreet pinching in the trapezoids is enough to produce significant pain. As we shall see later, any pressure is in fact allodynic and the non-specific points are simply those which are the most sensitive.

ADDITIONAL EXAMINATIONS, DIFFERENTIAL DIAGNOSIS

Additional examinations are essential for making a definitive diagnosis (20). Early diagnosis can improve patients satisfaction. Therefore, an initial comprehensive evaluation to exclude other conditions may help patients to realize their conditions and reduce uncertainty. A biological work-up is also essential. The results can vary from one patient to another, and from one doctor to another (21). The results may be normal, thus making it possible to eliminate other differential diagnoses. It is also possible to propose, systematically and as routine, a blood count, sedimentation rate, and C-reactive protein test so as to eliminate an inflammatory condition, protein electrophoresis to eliminate pathologies of the M-spike type or gamma globulin disorders, serum calcium, and phosphorus levels to eliminate hyperparathyroidism or osteomalacia, creatine kinase to make sure not to miss a muscle disease, and thyroid-stimulating hormone is essential for eliminating dysthyroidism. Depending on the context, if chronic inflammatory rheumatism is suspected, the decision can be made to screen for antinuclear antibodies, a rheumatoid factor and anti-CCP antibodies for rheumatoid arthritis, anti-thyroid peroxidase antibodies for Hashimoto's disease, anti-neutrophil cytoplasmic antibodies to make sure vasculitis is not missed, and HLA B27 if spondyloarthritis is suspected. If the progression is semi-recent and can be counted in months, other serological, virological or other tests may be useful, depending on the context. We can also cite the cytomegalovirus, Epstein Barr and herpes viruses, toxoplasmosis, Lyme disease, chikungunya and dengue fever in case of travel to tropical regions, as well as trichinosis and brucellosis which are nevertheless exceptional if there is no travel to countries where these conditions are endemic. Radiology and imaging examinations are said to be normal, though it would be better to say that there are no specific elements. Depending on the degree of painful discomfort, it may be useful to suggest a pelvic X-ray to look at the sacroiliac joints, X-rays of the lumbar, dorsal, and cervical spine to identify any arthrosis or degenerative disc disease, but with non-specific ordinariness, X-rays of the shoulders and hips in search of apatite calcifications, which can also affect both belts, as is predominantly the case with fibromyalgia pain. Finally, an X-ray of the thorax can be justified to diagnose sarcoidosis. Other additional examinations are often requested out of excess, even if it is possible to need imaging to screen for bone or enthesitic lesions; an electromyogram to screen for radiculopathies, entrapment syndrome or polyneuritis; an ultrasound to identify synovitis, tenosynovitis, bursitis or tendinitis; a CT scan or MRI, often axial although degenerative conditions identified in this way are often misleading because they are very common and asymptomatic, such as osteoarthritis, degenerative disc disease and calcifications.

This long list of differential diagnoses, classified by organ (**Table 1**) summarizes the work of the diagnostician who aims to screen for and eliminate all them all, first of all clinically of course, and with the help of any appropriate additional examinations. It is possible to highlight the four pathologies that pose the greatest diagnostic difficulties in everyday practice: osteoarthritis, spondyloarthritis, dysthyroidism, and apatite-induced arthropathy. Muscle, bone, neurological and endocrinal workups can complete this diagnostic inquiry. But what really matters is that the practitioner has acquired a strong degree of confidence with the diagnosis of fibromyalgia, as this will also allow the patient to obtain the same diagnostic confidence. It has been shown even though the practitioner may request fewer additional examinations, the patient was less stressed and felt better (22).

PSYCHOLOGIC PROFILE

Understanding the psychological profile of fibromyalgia patients is one of the keys to better understanding the condition. The first question that needs to be asked is very simple: is fibromyalgia a matter of rheumatology, psychiatry, or algology? As we have already seen, rheumatologists play a key role at the diagnostic level. The psychiatry profile of fibromyalgia has been clearly defined, and it conditions how it will be dealt with subsequently. Finally, algology is a discipline involving natural management of this pain, with a multi-disciplinary approach given this level of multi-dimensional complexity.

TABLE 1 | List of differential diagnoses to be eliminated by the rheumatologist in cases of diffuse pain.

Muscle pathologies	Myositis Myopathy
Joint pathologies	Osteoarthritis Spondyloarthritis Rheumatoid arthritis Other form of vasculitis
Bone pathologies	Osteomalacia Fracturary osteoporosis Secondary bone localizations
Neurological pathologies	Myelitis Polyradiculoneuritis/polyneuritis Amyotrophic lateral sclerosis
Endocrinal pathologies	Hypothyroidism Hyperthyroidism Hyperparathyroidism
Viral pathologies	Cytomegalovirus Epstein Barr Herpes virus Chikungunya Dengue
Miscellaneous	Apatite-induced rheumatism Sarcoidosis Chronic Lyme disease Chronic toxoplasmosis Trichinosis Chronic brucellosis

From the concept of neurasthenia to that of fibromyalgia, a great many things have been said about the psyche of fibromyalgia patients. First of all, it is once again necessary to lay to rest the stigma of fibromyalgia as some kind of hysteria or pithiatic condition. There are, of course, fibromyalgia conversion disorders, but no more than in a control population (23). Let us now turn to more classic psychological profiles: depression, anxiety, stress, cognitive disorders, plus a few words about sexuality, before talking at greater length about the notions of coping, resilience, catastrophizing, and post-traumatic syndrome. It should be noted that all these psychological elements are highly variable from one fibromyalgia patient to another, with a very clear correlation between the intensity of the pain and the psychological profile.

Depression is a classic disorder found in ∼1 patient in 4, which is 2–3 times higher than in a control population (24). The main question is to know whether this depression is primary or secondary. Its importance has almost certainly been overestimated, and we can thus consider it normal that a woman who has been in pain for several years, with multiple questions and no reliable treatment response, can have a certain degree of relational depression. It should be noted that the depression is more marked when the pain is intense, and that depressed fibromyalgia patients are generally younger, with more pain, more sleep disorders, less social satisfaction, less well-being and a greater need for assistance (24).

The same observation can be made for anxiety and stress: there is the same prevalence of approximately one third, the same probably reactional origin, the same overestimated importance because it is expressed with insistence by fibromyalgia patients, and the same correlation between pain, anxiety, and stress (25).

Cognition is undoubtedly altered in fibromyalgia patients, whether it is attention, reasoning, memory, learning, perception, or recognition (26). The cognition disorders are associated with the symptom of fatigue, which is significant, with even sometimes exhaustion. This poor cognition is not only self-reported, but also objective when evaluated with neuropsychological measurement. Fibromyalgia patients have up to half of the short-term or long-term memory when compared to an age-matched control group (27). The cognitive impairments are correlated negatively with the degree of pain, but also with affective factors, as catastrophizing, low self-esteem and alexithymia (28).

Surveys have clearly shown that there are sexual disorders in fibromyalgia patients (29). With this, there are two approaches to adopt. The first is that although there is less sexual satisfaction, it is hard to imagine how anything else could be the case in these patients who not only endure diffuse pain, but also endure pain that is exacerbated by the slightest touch, without forgetting the gynecological points which are not spared by the allodynia. However, fibromyalgia patients have little or no alteration at the level of their desire or pleasure, even though there is no consensus in the studies on this subject. A second approach is also important. Sexual abuse can be found in the past history of 20–50% of fibromyalgia patients, either in childhood or as adults. The relative risk of finding sexual abuse in relation to a control group is 2.5–3.1 for fibromyalgia patients (30). This notion needs to be placed in the context of post-traumatic syndromes.

The term "coping" is often used, from "to cope," which comes from the French *couper*, to cut, separate, hit. And we find that in expressions like "cut to the quick." The meaning of coping, though, can be summarized in this definition: all the permanently changing cognitive and behavioral efforts that an individual uses to respond to specific internal and/or external demands, evaluated as very high and going beyond his or her adaptive resources (31). The term is associated with the notion of resilience developed by Boris Cyrulnik. In certain people, the default "coping" strategy, with difficulty adapting to a complex situation, will lead to a psychological disorder: dissociation, sensitization, safety attitude, avoidance, or self-medication. We can immediately see the association with a causal factor, the consequence of a post-traumatic stress syndrome. The psychometric evaluation can be made using the Chronic Pain Coping Inventory-42 (CPCI-42) or the Coping Strategy Questionnaire (CSQ). In fibromyalgia patients, the coping is deficient. Thus, in difficult situations, and here we can cite for example a bombing, sexual abuse, a natural catastrophe, war, or holocaust. But much less extreme events can be traumatic in these deficient coping patients who develop fibromyalgia. They will not be able to find solutions that allow them to move forwards, and turn in circles around the traumatic event.

The second important term to define the profile of fibromyalgia is "catastrophizing." This is first and foremost a pessimistic attitude, negativism, alarmism, someone who sees everything as black and always sees the worst in a situation and is tied to disaster (31). It can be evaluated using the Pain Catastrophizing Scale, or the Sullivan Scale. The

notion of trauma, in the broadest sense of the term, is once again significant. The same catastrophe situations that generate "coping" can be associated with the notion of "catastrophizing," as for example in climate changes, conflicts and wars, natural catastrophes and possibly viral pandemic in the future.

Catastrophizing is an important pattern in fibromyalgia, clearly associated with pain intensity, provoked pain (9). Nevertheless, functional magnetic resonance imaging did not found any association between the assessment of an experimental provoked pain and catastrophizing, as well as for anxiety or depressive symptoms (32). There were no correlations with the duration of the fibromyalgia. If the pain sensitivity is increased in fibromyalgia, its neural mechanism seems in part different from the negative mood affects, as depression, anxiety and catastrophizing. But high catastrophizing in fibromyalgia could take part in a decrease of the pain inhibition which occur during distraction cognitive tasks, in a model of thermal stimuli with functional MRi assessment (33). This mechanism could contribute to explain the pain and the other symptoms persistence, with the need of interventions to reduce catastrophizing, as cognitive behavioral therapy.

Psychological trauma plays a very well-defined role in the genesis of fibromyalgia, with prospective follow-up studies such as the one by Lawrence-Wolff. In this study, on American Gulf War veterans, 40% had been in a war situation, and the diagnosis of fibromyalgia went from 2 to 8% on their return, with a quarter presenting post-traumatic syndrome (34). Patients at risk had already suffered from pain in the past (relative risk × 2.4), but above all catastrophizing (relative risk × 11). Traumatic events can also be physical, associated with a secondary psychological impact. A prospective, 2-year follow-up study on patients admitted to the Emergency Room after a car accident in London and Toronto is the perfect illustration (35). It was possible to identify three groups: 383 serious accidents but with no cervical impact; 224 accidents involving cervical trauma; and 643 minor accidents. None of these patients had suffered pain before. In relation to the reference group—minor accidents—the traffic accidents developed almost 5 times more cases of fibromyalgia at 2 years, with a relative risk of 5.2, and a rate of 3% when there was a cervical impact, with a relative risk of 8.4. We can see here the important role played by trauma and the cervical location, which is a source of anxiety given the potential seriousness.

Professional problems are important, with repeated periods of sick leave which often inspire a lack of understanding given the organic mildness. But the long duration of the symptomatology, plus the personal, professional, and family desocialization can deserve a better attention (36). Associations for fibromyalgia patients have thus played a part in improving understanding of the condition in both the general public and the medical profession, and thus recognition of an affection that is not "made up." Alongside these positive actions, requests for classification of the condition as chronic long-term illness and handicapped status for all, the quest for a miracle drug or a perfect diet that does not exist, as well as belief in sometimes doubtful theories developed by some, all still have a negative impact on the credibility of fibromyalgia patients, who are nevertheless barely affected by this contestation. Finally, a precise psychological diagnosis and the psycho-social impact are important to be evaluated, in order to offer adapted psychological interventions, which are a major point of the therapeutic approach (37).

TABLE 2 | FIRST questionnaire to identify fibromyalgia within a population.

6 selected questions
1. My pain is localized all over my body
2. My pain is accompanied by permanent general fatigue
3. My pain is like burning, electrical discharges, or cramps
4. My pain is accompanied by abnormal sensations, such as tingling, pins and needles or numbness, all over my body
5. My pain is accompanied by other health problems such as digestive disorders, urinary tract problems, headaches, or restless legs
6. My pain has a significant impact on my life: in particular, on my sleep, my ability to concentrate, giving me a feeling of being in slow motion

FIRST positive if ***≥ 5/6*** *of the items present.*
Sensitivity 90% and Specificity 86%.

DIAGNOSTIC AND EVALUATION CRITERIA, PROGNOSIS FACTORS

The FIRST questionnaire makes it possible to detect a target fibromyalgia patient population (38). These 6 statements are expressed as being the most specific for the experience of fibromyalgia patients (**Table 2**). If patients recognize themselves in at least five of them, there is a 90% chance that they have fibromyalgia.

After the initial diagnostic criteria dating back to 1990 (19), with a low degree of specificity and putting the highlight on pain points, Yunus and Wolff developed new diagnostic criteria in 2010, revised in 2016 (39, 40). The 2010 diagnostic criteria were composed of 2 chapters. One listed the spontaneously painful points, the other the associated symptoms. The 19 pain points were reduced to 15 in 2016, but nevertheless they still focused much too heavily on peripheral pain (12 of the points) in relation to axial pain (3 points), when the hierarchy in relation to their respective importance is the opposite (**Table 3**). The associated symptoms are divided into four chapters: fatigue, non-restorative sleep, cognitive disorders, and other associated symptoms. They remain rather unspecific. The diagnosis is confirmed on the one hand if there are at least seven pain points with an associated symptom score of at least five. Thus, for example, pain in the shoulders, hip, and spine, combined with fatigue and fragile sleep patterns associated with some attention deficit disorders would satisfy the criteria. It is nevertheless easy to see how a differential diagnosis might be missed with diffuse pain of other origins. The notion of absence of another diagnosis is thus very important. The 2016 criteria, however, added that there are sometimes associated, making the diagnosis extremely difficult. The 2nd diagnostic approach has very few pain points, essentially the spine, and a wide range of associated symptoms. The 2016 revision requires at least a 4th pain point (39). These diagnostic criteria can be used not only in studies, but also when one asks questions about a sure diagnosis of fibromyalgia because they

TABLE 3 | ACR 2010 diagnostic criteria, revised in 2016.

Widespread pain index (WPI) (pain > 3 months) (circle the areas that were painful during the past 7 days)				
	Neck			
Right shoulder				Left shoulder
Right upper arm				Left upper arm
Right forearm				Left forearm
	Upper back			
Right hip/buttock				Left hip/buttock
Right upper leg				Left upper leg
Right lower leg				Left lower leg
	Lower back			
Calculation of the number of painful areas: TOTAL ☐ …out of 15				
Severity of the symptoms (SS) during the past 6 months [from 0 (none) to 3 (severe)]				
Fatigue	0	1	2	3
Non-restorative sleep	0	1	2	3
Cognitive disorders	0	1	2	3
Severity of the associated symptoms	0	1	2	3
Calculation of the severity of the symptoms: TOTAL ☐ …out of 12				
Positive ACR diagnosis				
	if	WPI ≥ 7 and SS ≥ 5		
or	if	WPI 4–6 and SS ≥ 9		
and	if absence of other diagnosis			

But sometimes a possibility of an associated condition.
Sensitivity 88% and Specificity 81%.
To be completed by the doctor.

provide sensitivity and diagnostic specificity values of 80–90%, which is very acceptable.

In studies that assess fibromyalgia, in addition to pain and fatigue type visual analog scales, the FIQ questionnaire, revised in 2009, makes it possible to obtain an overall approach on the progression of the fibromyalgia (41, 42). It has thus become essential in the various studies assessing fibromyalgia. Ten per cent of the questionnaire is associated with function, 20% with the number of days without activity, and 70% for difficulties encountered in professional and everyday life. Fibromyalgia is located at scores of around 50–70 out of 100, depending on the severity.

The prognosis factors are associated with the severity of the symptomatology, be it the fatigue, pain, associated signs, or the global index, the FIQ (31). But the psychological factors are also prognostic, be it the depression, anxiety, stress, or realm of catastrophizing (25). There is no over-risk of mortality in fibromyalgia (43). As for the progression in the mid-term, there is little to no change at 1 or 2 years (44). The long- and very long-term progression is poorly known, but it can be noted that there are few or even no cases of fibromyalgia after the age of 70 years, even if the condition has existed for a long time. A small cohort monitored at 26 years revealed that 11% of the fibromyalgia cases had been cured and 23% had remission lasting more than 1 year (45). But a recently published study revealed that the risk of suicide is far from negligible. A major meta-analysis of almost 400,000 fibromyalgia patients very clearly revealed more suicidal thoughts, with a relative risk multiplied by 9.1, and suicide attempts, with a relative risk multiplied by 3.1 (36). Of the other risk factors, we can mention professional status, the severity of the fibromyalgia (whether via the VAS for pain or the FIQ), chronicity, obesity, and addiction to opioids. We cannot stress the last factor enough as it is one of the "big affairs" in drug prescriptions in recent years, with, it should be remembered, a mortality rate of 30,000 people a year in the United States. Finally, there is always the psychology of these patients, with depression, anxiety, mental health, and sleep disorders. Fibromyalgia should thus be taken seriously by screening in severe cases for these risk factors of suicide, and screening for profound depression associated with the severe cases, plus addiction to opioids or products such as ketamine which can disinhibit and provoke a dissociative effect.

ETIOPATHOGENIC DEBATE: PAIN, SLEEP DISORDERS, AND FATIGUE

Here are a few questions for which we can try and provide some elements of response. What is the reality of the pain, with peripheral explorations and the role of neurotransmitters yet to be defined? Where does the pain come from, with notions of hyperalgesia and allodynia, the notion of pain thresholds and pain amplification? What is the role of fatigue and sleep disorders, and what possible role of induction do they have?

Substance P and glutamate, secreted by nociceptive neuronal fibers, have been the subject of unambiguous studies, with high concentrations in the CSF of fibromyalgia patients in relation to controls, in a non-specific manner, and with normal serum concentrations (46). The same is true for neurotrophins of the NGF or BDNF type (47). Enkephalins may be good candidates. Naloxone has given disappointing results, and may provoke diffuse pain, like morphine (48). Endorphin concentrations in the CSF of fibromyalgia patients remain normal, and as we have already seen, morphine is a disappointing treatment for fibromyalgia pain (49). Similarly, a study using the PET scan with Carfentanyl, which binds to endogenous μ-opioid receptors in the regions of the central nervous system devoted to pain, found a decrease in the signal in 17 fibromyalgia patients in relation to 17 controls (50). There is thus an inverse correlation between the intensity of the pain and binding at the level of the nucleus accubens, stratum and cingulate cortex. The hypersensitivity to pain in fibromyalgia patients is in contrast to diminished activity in the central nervous system and explains the disappointing results of opioids when treating fibromyalgia pain. In addition, the risk is an increase in doses which can lead to addiction and toxicity, with all the dramatic consequences and mortality that that implies.

Serotonin could have been the key neurotransmitter in fibromyalgia. It effectively modulates nociceptive information at the supramedullary level, it regulates sleep and is associated with the presence of slow waves. There are different sites and brain concentrations in men and women, and it is modulated by estrogens (51). Thus, in fibromyalgia, concentrations of

serum tryptophan, the precursor for serotonin, are decreased. There is polymorphism in the serotonin receptors. Serum serotonin concentrations are inversely correlated with the severity of fibromyalgia, and concentrations of 5 HIAA in the CSF, the metabolite of serotonin, are decreased. However, the serotonin re-uptake inhibitors are not every efficient on the pain of fibromyalgia. That is more the role of serotonin and noradrenaline re-uptake inhibitors. The neurotransmitter approach and fibromyalgia can only be envisaged by associating several together, not just serotonin, but also GABA, dopamine, and noradrenaline (51).

The allodynia is not limited to the standard pain points of fibromyalgia. The pressure of an armband on the muscles in the arm is thus more painful in fibromyalgia patients, with a pain threshold that is ∼20–30% lower than in a control group (52). In addition, allodynia is the rule in fibromyalgia 7 times out of 10, whereas it is rare in arthrosis, rheumatoid arthritis and in controls with no **illness**. This hypersensitivity is correlated with the parameters of progression of fibromyalgia. Hyperalgesia and allodynia are thus generalized, associated with an alteration to the pain threshold. But this decrease in the threshold for pain is not only related to painful pressure. A study also tested, in addition to pressure pain, heat, cold and stings (53). The decrease in the receptiveness threshold was around 20%, except for cold, where it was actually more than 100%, during or after the stimulus.

This mechanism can be explained by the fact that in normal physiological situations, whether we are standing, sitting, walking or even lying down, we always have a certain muscle tone, a certain tension in our tendons and muscles, and stimulation of the receptors, even at room temperature. There is thus a certain amount of nociception which is activated and stimulated permanently. But when this nociceptive signal level is low, the brain integrates the signals as normal and does not emit any information of the pain type. Among these hypotheses, we can point the finger either centrally, with the collapse of the pain threshold, or an amplification mechanism for this normal signal, or alteration to the retrocontrol mechanisms of the inhibitor system. In any case, the result is the same, with amplification of the pain signal felt, which can be illustrated as the volume control button on a music system. Finally, the response time for pain is lengthened, just like the duration of the response felt to pain. The same amplification mechanism can be felt after sensory stimuli with quantitative measurements in functional MRI, whether the stimuli are visual, auditory, temperature or sensory (54). The intensity of the signal is correlated with the intensity of the pain and the functional handicap induced.

Along with the pain, fatigue is the other major element in the clinical picture that must be highlighted in fibromyalgia. One study illustrates this particularly well: 87 adults who practiced sport and had no sleep disorder were randomized into four groups: controls, restricted exercise, restricted sleep or restricted exercise and sleep (55). By the 10th day, symptoms equivalent to those found in fibromyalgia started to appear in restricted sleep groups: pain, major fatigue, and thymus and cognition disorders. It should be noted that exercise had a certain inhibitory effect in men, and that fatigue played a preponderant role in women. In personal practice, everyone has been able to observe that after periods of troubled sleep, or a sleepless night, or a viral syndrome, there is generalized soreness or aching, associated with the induced fatigue.

Sleep disorders are thus almost always mentioned. They are real, as observed by polysomnography (17). It is possible to observe decreased sleep duration, altered sleep quality with more phase 1 and fewer slow waves, and an increase in the number of nocturnal wakings. These anomalies can be quantified using the Pittsburg Sleep Quality index (56). There is real dissociation between the sentiment of sleep disorders and the objective data. What remains is that while this non-restorative sleep certainly plays a role in understanding the physiopathogenesis of fibromyalgia, it has not so far resulted in drug therapy measures that are genuinely effective, with mitigated results with melatonin and disappointing results with agomelatine, a melatonin agonist (57, 58).

The kinetic analysis of events is important to better understand their role in the etiopathogenesis of fibromyalgia (**Figure 1**). In the follow-up to post-traumatic syndrome, regardless of the trauma, in certain fields particularly associated with the persistence and non-resolution of problems, with a more or less catastrophist or negative vision of things, the psychological disorders induced are associated with sleep disorders. The result is chronic fatigue, associated with hyperreactivity and overall hypersensitization, with amplification phenomena in the sensory signals that are expressed loudly by persistent, invalidating pain at rest. All feelings are hypertrophied, regardless of the sensory or psychological nature. We can observe that chronic fatigue precedes, often by several years, the onset of the pain and all the associated functional signs.

ETIOPATHOGENIC DEBATE: NEURO-IMAGING, SMALL FIBER NEUROPATHY, AND EPIGENETICS

Modern imaging methods, as PET scan combined with functional scintigraphy or functional MRI, have been possible to identify the structures involved in central integration of pain. The well-known entry point for pain is the thalamus. It sends multiple connections toward the cingulate cortex, the sensory cortex, and the insula. All these regions send information on sensory discrimination, intensity, and the affective and psychological aspect of the pain. The diagram of these interconnections is of still poorly understood complexity, in a global dynamic process, with input, output, integration, memorization and modulation that we are only just starting to understand (59). Functional brain localizations have been identified for the catastrophizing, coping and depression processes, although there is no correlation between these identified psychological factors and the measures made in functional MRI and hyperalgesia (60). Prefrontal integration of pain is associated with catastrophizing. Pain is thus modulated in relation to fears, beliefs, and isolation, with integration defects.

The volume of cortical gray matter has been described as decreased in fibromyalgia (61). However, it cannot be explained by a neurodegeneration, with an upregulation of the $GABA_A$

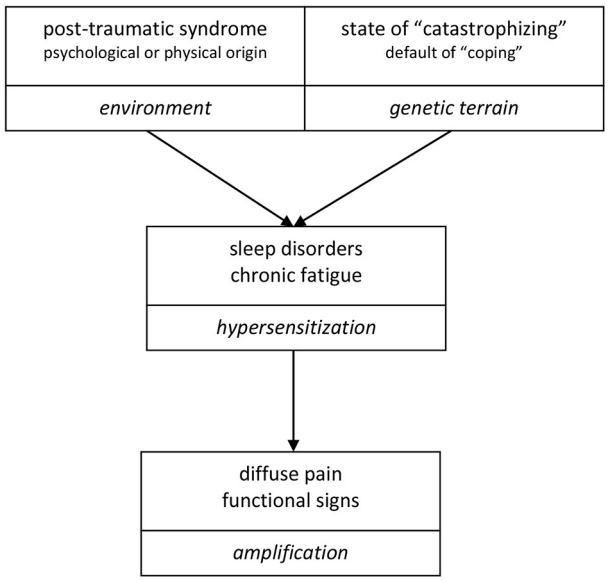

FIGURE 1 | Respective role played over time of the psyche, sleep disorders, fatigue, pain, and sensory functional disorders: kinetic analysis of the events occurring in the life of a fibromyalgia patient. The traumatic psychological events are old, or even dating back to childhood. The sleep disorders are secondary and quickly associated with a state of fatigue, which precede often by several years the chronic pain state and associated functional signs, in the sequence illustrated below.

receptors in MRI imaging, which could reflect an increase in neuronal matter. Throughout a person's life, neurons have the ability to modify their connectivity and activity in relation to our environment, our epigenetics, and also in relation to our genetics. The same is true with regard to pain (62). The number of neurons involved in pain is < 5%, but, in situations of chronic pain, this number rises to 15–25%. Many other cortical regions are involved in modulating the integration of pain. It has been observed that it is necessary to make use of this neuropathic pain and circumvent the neuronal neo-circuits that amplify the pain and cause it to last over time.

In 2017, Lopez-Sola published a definitive article with regard to the search for a real, measurable diagnostic signature in neuro-imaging with functional MRI (63). When a peripheral signal is emitted, it is possible to measure it at the level of certain electively involved regions. But what should we be measuring, given that it has to be something quantitative? A photo of mountains illustrates the results obtained in functional MRI, with peaks and valleys between the different regions measured. It was decided to quantify the signal by capping the activity peaks, with a reproducible manner. The study included 37 right-handed fibromyalgia patients vs. 35 controls paired for age, sex, and socio-educational level. Functional MRI was carried out after nociceptive stimulation to pressure of the thumb, and after neurosensory, visual, auditory and tactile stimulation. Hyperalgesia was found in the fibromyalgia patients in relation to the controls, with amplification of the pain signal of around 50%. A statistical mediation model made it possible to verify that the quantitative measurements in functional MRI truly reflected the variations in pain and unpleasant feelings experienced by the patients. In the first stage, a functional MRI was carried out after nociception with pressure at 4.5 kg/cm^2. It was thus possible to measure the signal in the regions generally involved in the reception of the nociception of pain, whether this was at the level of the thalamus, somato-sensory cortex, insula, operculum, or anterior cingulate cortex. These measurements were also carried out in the neighboring regions. An increase was observed in the central signal in these regions in relation to the controls, corresponding to an increase of one third of the pressure if the pressure went from 4.5 to 6 kg/cm^2 in the controls. The ability to make the fibromyalgia—control distinction, as a classification, was 68%. The same methodology was assessed by means of functional MRI at the level of the regions of the brain involved in modulating nociception: the perigenual posterior cingulate cortex, paracentral lobule and precuneus. It was thus possible to study the sympathetic, affective, self-referential, decisional, and sociocultural valences of pain. The difference between the fibromyalgia patients and the controls was very clear, and even more discriminating. The classification power of this second method was 71% between the controls and fibromyalgia patients. A 3rd stage, still following stimulus via painful pressure, measured the weight of the three representative regions by evaluating their ability to distinguish controls from fibromyalgia patients, as much on the positive signals as on the negative ones. Once again, it was possible to make the distinction and a classification at 70% between the two populations. The 4th stage was carried out following sensory stimulus. This stage measured the weight of four representative regions by assessing their ability to distinguish controls from fibromyalgia patients, as much on positive signals as on negative ones, with regard to visual, auditory, and tactile stimuli. The classification capacity was even better, at 89%. Finally, if all four of these stages were combined, the respective regions for the nociception of pain, the weight of the representative regions for pain, and the sensory stimulation, the static analysis revealed a classification capacity of 93%, distinguishing very well the fibromyalgia patients from the control population, with sensitivity of 92% and specificity of 94%. Associations were found between the increase in the brain signal of fibromyalgia patients in functional MRI and pain, sensory stimuli, the FIQ and depression. On the contrary, no association was found between the duration of progression of the fibromyalgia, and the consumption of antidepressants or anxiolytic drugs. There remain many other fields of investigation using this technique with, as a perspective: distinguishing fibromyalgia patients from other forms of chronic pain, assessing the differences between the various hypersensitization syndromes, studying the localizations associated with fatigue, post-traumatic syndrome and coping, and their respective roles, bearing in mind that depression and anxiety do not seem to play a preponderant role, and finally, to find a simple method for assessing fibromyalgia patients with functional MRI with diagnostic and prognostic aims. As we have seen, neuro-imaging has allowed us to make progress in the concept of fibromyalgia as a cognitive disorder of cortical integration of chronic pain. There is thus amplification of

the pain and sensory nociception signal, a decrease in the pain perception threshold, and persistence of the stimulus that maintains the process in chronicity.

A small fiber neuropathy has been reported with high prevalence in fibromyalgia. In a recent metaanalysis, a skin biopsy was positive in 45% of 176 patients (64). The diagnosis can be assessed with corneal confocal microscopy with even a better sensitivity. However, these findings are not at all specific, found in many pathologies, such as metabolic diseases, infectious diseases, chronic inflammatory rheumatism, toxic substances and genetic diseases. Finally, there is no correlation between this small fiber neuropathy and the somatosensory system function, so it does not seems to play a role in the pathogenesis of the fibromyalgia (65).

Epigenetics is also a new field of investigation in fibromyalgia (66). DNA methylation and miRNA expression are modified in fibromyalgia, with a role for environmental factors, such as stress, traumatism, sleep disturbance. While the correlations with symptoms are still weak, these explorations are only just beginning, and they could be of interest in the future for a better understanding of the diagnosis, persistence and treatment of fibromyalgia.

THE POLYMORPHISM OF PAIN

The pain of fibromyalgia is polymorphous and very rich: hyperalgesia, allodynia, hyperpathia, diffuse and variable pain, paresthesia and dysesthesia, with a wide range of varied feelings, including pseudo-algodystrophy, burning, pseudo-arthritis type swelling, tingling, numbness, stinging, pseudo-neurogenic, pseudo-entrapment, and pseudo-muscular or abdominal cramps, spasms and tightness with digestive, vesical, thoracic and gynecological symptomatology. The pain of fibromyalgia can affect every structure in the musculoskeletal system, including the tendons, ligaments, entheses, muscles, joints at the level of any region, above all axial and spinal, but also peripheral, as well as dental, temporomandibular and thoracic for example. Finally, the pain of fibromyalgia can also affect the deep organs, with pain that can be digestive, with epigastralgia and reflux, pseudo-colitis, pelvic pain, vesical pain, headaches, with all the symptomatology contrasting with negative or non-significant additional examinations (67).

There is thus no specificity in the pain of fibromyalgia. Fibromyalgia can be perfectly in adequation with the evaluation scores for other specific pathologies, such as the DN4 for neuropathic disorders, and the BASFI and BASDAI for spondyloarthritis, for example (68). Many diagnostic criteria can be assessed positively in fibromyalgia, such as those for rheumatoid arthritis, but above all for spondyloarthritis, with SSAS criteria that are often positive and, for the latter, a diagnostic problem that remains difficult to solve between female spondyloarthritis and fibromyalgia (69).

Fibromyalgia is sometimes used as a catch-all term to label any condition that is painful but that is not fully understood. And this naturally depends on the rheumatological culture, whether or not we fully master the diagnostic elements, for spondyloarthritis, apatite-induced rheumatism, osteoarthritis, and fibromyalgia. It also depends on the rigor in the initial workup, sometimes skeletal, sometimes even an enormous case file to wade through, with five MRI and three CAT scans, but no thyroid-stimulating hormone. One must therefore be careful not to blindly trust fibromyalgia diagnoses made too easily and in excess. On the contrary, fibromyalgia is still sometimes denied—< 20 years ago, it is true—but still there are colleagues who believe that something that is not well-understood cannot exist, in a sort of exacerbated hyper-rigorism and scientism. There are still also incorrect opinions, classing the condition as hysteria or pithiatic, with a certain amount of ignorance and reductionism of the complexity of the psychiatry. These attitudes still lead to insufficient diagnoses, medical nomadism, and high costs for fibromyalgia.

THE ASSOCIATED SYMPTOMS, OTHER SIMILAR PATHOLOGIES, ASSOCIATED PATHOLOGIES

Yunus has put together a very long list of the symptoms associated with fibromyalgia pain (70). We can classify them in three groups: the very common, the common and the less common. At the top of the list of very common associations we find chronic fatigue. This symptom can even make us review the diagnosis if it is absent. It is increased when activities are stopped and decreased with exercise. It is correlated with depression, sleep disorders, the intensity of the pain and hyperalgesia. It is also very clearly inter-related with chronic fatigue syndrome, a "sister" **condition**. Sleep disorders are also almost constant, with difficulty falling asleep, non-restorative sleep and light sleeping, and frequent waking. They are associated with emotional disorders, gastro-esophageal reflux, loss of urine and dyspnea, and may be related to sympathetic hyperactivity.

Among the common symptoms that are associated in about one case in two, paresthesia and dysesthesia are correlated with the pain, but not with psychological disorders. They can resemble a more neurological picture such as multiple sclerosis or amyotrophic lateral sclerosis, or a pseudo-entrapment syndrome. Feeling of bloating is common. Headaches resemble migraine, and neurological investigations remain negative. The dizziness is not rotary, with ear explorations also negative.

Of the less common associations, found in roughly one in three cases, we can note dryness in the mucosal membranes, particularly in the mouth, and in the absence of any iatrogenicity, with negative explorations, which sometimes be led to perform a biopsy of the saliva glands; cold extremities, and once again the absence of any iatrogenicity and negative explorations, particularly Doppler; dysmenorrhea, with pelvic pain and sometimes headaches, and once again negative explorations; tinnitus with negative ear explorations; and finally cognitive disorders such as attention deficit, memory or information processing disorders specific to chronic fatigue.

There are also pathologies, often known as functional, that remain close to fibromyalgia (66). We can highlight chronic fatigue syndrome, also similar to "burn out," which is a very

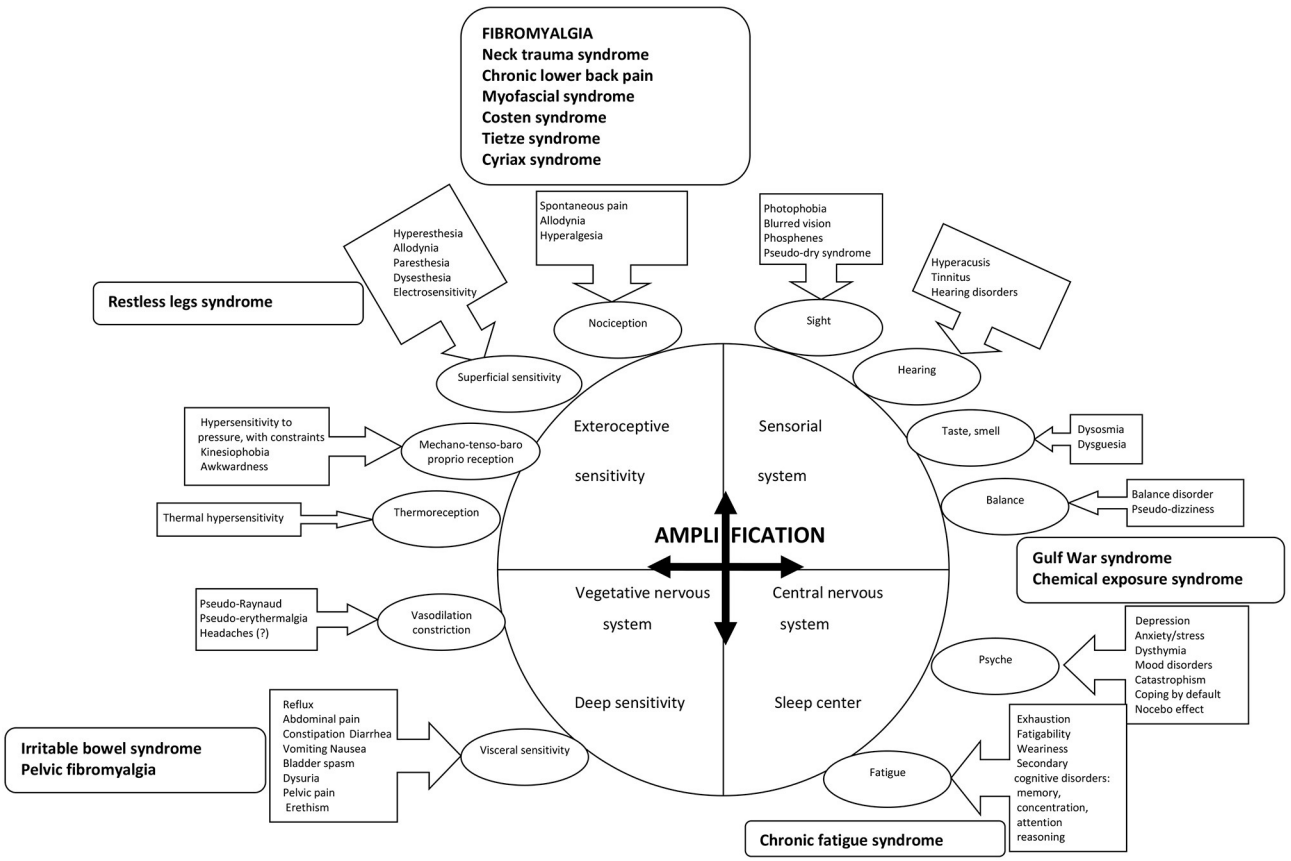

FIGURE 2 | The different paths to the origin of the stimuli responsible for the symptomatology in fibromyalgia are illustrated in the bubbles, with the associated signs in the boxes linked by arrows. The stimuli at the origin of the associated symptoms of fibromyalgia are either of exteroceptive origin, or profound, or related to the neuro-vegetative system, all with medullary relays, or in relation to sensoriality and its bulbar relays, or even central origin with regard to the psyche or fatigue. Each time, there is chronicization and amplification of the signal, which can thus only be done at the central level. The supposed mechanism is either abnormal amplification of the signal itself, or inhibition of the retrocontrol system, or a quantitative decrease in the perception threshold for the signal. A few entities (in bold in the boxes with rounded corners) are the subject of specific names in relation to the more specific predominant symptomatology, but with clear ties with fibromyalgia in the context of the hypersensitization which is common to all these syndromes.

common privileged association, with major, unexplained fatigue as the main common point. Algo-dysfunctional syndrome of the manducatory tract, accompanied by negative stomatological examinations, is also very common. Restless legs syndrome, in one in 3 cases, is associated with abnormal nocturnal movements. Tension headaches, caused by abnormal tension, can be the subject of a request for a brain MRI, which is without particularity. Irritable bowel syndrome, with bloating and alternating diarrhea and constipation, produces a normal coloscopy and presents with many similarities with fibromyalgia, as it also combines headaches and chronic fatigue; it can be treated with duloxetine, like fibromyalgia. Myofascial pain syndrome is accompanied by regional pain with trigger points of a functional nature. Chemical exposure syndrome is a mini-psychosis based on exposure to a supposedly toxic product or a product supposed to be present, combining headaches, nausea, dizziness and pain. It is also possible to make connections with Gulf War syndrome, particularly with the fear of having been exposed to radioactive products even though the in-depth studies carried out have not found any particular cause for the extremely rich and varied functional symptomatology. Pelvic fibromyalgia and the eight pelvic pain points described has led to reports of surgeons performing excessive numbers of unexpected hysterectomies on the basis of the pain alone, even though the other examinations were negative. At the level of the spine and thorax, we can note neck trauma syndrome following a whiplash injury—the diagnosis of a mild sprain is made after negative imaging results yet the pain, which generally lasts for a few weeks, instead lasts for several months or even years. Certain forms of chronic refractory lower back pain are also considered to be the equivalent of fibromyalgia, often following an effective spinal episode but that lasts for months or even years. Tietze and Cyriax syndromes associate sterno-costal pain and slipped ribs, which again last for months and months, without ever being able to find any organic condition.

Finally, all these "sister pathologies" to fibromyalgia can in fact be associated with each other (57). Fibromyalgia can be associated with all of them. Chronic fatigue syndrome can

too, with irritable bowel syndrome, tension headaches, Costen's syndrome. Irritable bowel syndrome can be associated with fibromyalgia and chronic fatigue syndrome, but also with tension headaches and pelvic fibromyalgia. Migraines can be associated with restless legs syndrome. And chemical exposure syndrome can be associated with chronic fatigue syndrome and irritable bowel syndrome.

Other organic pathologies have a privileged association with fibromyalgia. The first of which is feminine spondyloarthritis, which as we have seen is a difficult differential diagnosis (69). Female spondyloarthritis has a mean delay of diagnosis between 7 and 9 years, despite the technical means at our disposal, such as MRI. The condition results in little stiffness from a clinical or radiological point of view, and there is a very similar symptomatology—axial—often with a nocturnal pain and chronic fatigue. This association is found in 10–30% of cases of spondyloarthritis (69, 71). It is necessary to determine what proportion of the symptoms can be attributed to each of the two pathologies. Care must be taken regarding the use of biotherapies as they are not relevant if fibromyalgia predominates, even though the more the spondyloarthritis is in flare-up phase, the more the fibromyalgia will be painful. It is also necessary to remember this if biotherapy fails in a case of spondyloarthritis. Fibromyalgia amplifies chronic pain and is a factor in its persistence of chronic painful osteoarthritis of the spine, particularly if there are multiple localizations, cervical and lumbar. It could be a difficult differential diagnosis in women because the symptomatology is similar. In one in three cases of fibromyalgia, Gougerot-Sjögren syndrome is associated with pain that must be differentiated from genuine inflammatory myositis. Autoimmune thyroiditis, or Hashimoto's disease, is rarer, but is also associated in roughly one in three cases with fibromyalgia. Rheumatoid arthritis is less commonly associated with fibromyalgia—roughly one in ten cases. We can also note the role played by stress, depression, and "catastrophizing." Disseminated lupus erythematosus is associated in roughly 10–20% of fibromyalgia cases, and once again, the fibromyalgia pain must be dissociated from the genuine inflammatory myositis.

HYPERSENSITIZATION SYNDROME OF CENTRAL ORIGIN AND ETIOPATHOGENIC HYPOTHESES

At the basis of this concept of hypersensitization of central origin is the king of fibromyalgia in the 1980s, Yunus (72). Hypersensitization is well-known for pain. As thus are hyperalgesia, allodynia and the famous fibromyalgia pain points from the former criteria. In fact, allodynia and hyperalgesia are generalized. The points that are naturally the most sensitive in anyone are the same in a fibromyalgia patient and are also spontaneously painful. Entheses are structures that are very rich in nociception. But hypersensitization also implies a wide range of other fields, both sensory and psychogenic. Hyperalgesia is thus one criterion that must be taken with a great deal of caution and considered as one of the elements of generalized hypersensitization. Here are types of hypersensitization other than the pain found in fibromyalgia, and which have all been proven (72). Fibromyalgia patients are thus more sensitive to changes in temperature, either hot or cold, and this can be observed when they travel. As has been seen in functional MRI, there is hypersensitization to touch and pressure. Hypersensitization to injections has also been observed when it is necessary to take blood. Patients are also sensitive to ischemia (52). They dislike loud noises and harsh lighting (73). Finally, it has been observed that there is hypersensitization to electricity when an electromyogram is requested. Alongside this sensory hypersensitization, these patients are light sleepers and have side effects to drugs more commonly than other patients treated with the same drugs for other pathologies: at an identical dose, a fibromyalgia patient will have twice as many side effects (74). There is also a high degree of medical failure. It should be noted that all this hypersensitization other than pain can also be found in the sister pathologies to fibromyalgia. Some entities are individualized, with specific denominations, in relation to the more specific predominant symptomatology, as chronic fatigue syndrome, irritable bowel syndrome, neck trauma syndrome, pelvic fibromyalgia, chemical exposure syndrome, gulf war syndrome, myofascial, Costen, Tietze, Cyriax syndromes (**Figure 2**). Even some presentations of chronic low back pain are sometimes included in this list. All these entities are clear ties with fibromyalgia in a context of the hypersensitization, which is common to all these syndromes. **Figure 2** summarizes and collects all the associated symptoms common to the different clinical pictures for hypersensitization. It can involves exteroceptive stimuli or profound sensitivity, or the vegetative nervous system, all with medullary relays. The sensory system will have bulbar relays. The central nervous system itself is involved (fatigue, cognitive, and psychological troubles). The central level is responsible of the chronicization and amplification of all these signals, either by an abnormal amplification of the signal itself, or by inhibition of the retrocontrol system, or a quantitative decrease in the perception threshold for the signal.

CONCLUSION

Fibromyalgia is a cognitive disorder of cortical integration of pain. There is amplification of the nociception signal, both at the pain and sensory levels, with a decrease in pain thresholds. There is also persistence of a stimulus, which maintains the condition in a chronicity process. But fibromyalgia must be placed more broadly in the context of chronic hypersensitization syndromes of central origin, a context that is both complex and global, with a very wide range of means of expression.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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IL-33 in Rheumatic Diseases

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Interleukin-33 (IL-33) is a nuclear factor mainly expressed in barrier epithelium, endothelial cells, and fibroblast reticular cells. Some inflammatory cells also express IL-33 under certain conditions. The important role of IL-33 in allergic reactions, helminth infection, cancer, tissue fibrosis, chronic inflammation, organ transplantation, and rheumatic immune diseases has been extensively studied in recent years. IL-33 primarily activates various circulating and tissue-resident immune cells, including mast cell, group 2 innate lymphoid cell (ILC2), regulatory T cell (Treg), T helper 2 cell (Th2), natural killer cell (NK cell), and macrophage. Therefore, IL-33 plays an immunomodulatory role and shows pleiotropic activity in different immune microenvironments. The IL-33/serum stimulation-2 (ST2) axis has been shown to have a detrimental effect on rheumatoid arthritis, systemic lupus erythematosus, and other rheumatic diseases. Interestingly, IL-33 also plays a protective role in the repair of barrier epithelium and the activation of Tregs. Therefore, the role of IL-33/ST2 depends on the underlying pathological conditions in rheumatic diseases. This review focuses on the dual role of the IL-33/ST2 axis in rheumatic diseases.

Keywords: IL-33, alarmin, ST2, autoimmune, rheumatic disease

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INTRODUCTION

Interleukin-33 (IL-33), a member of the IL-1 family, was first discovered in human tissues in 2003 and was originally defined as a nuclear factor of high endothelial venules (NF-HEV) (1). In 2005, Schmitz et al. reported that the C-terminal (amino acids from 112 to 270) of NF-HEV exhibited an IL-1-like three-dimensional folding and induced a type 2 immune response through binding to its receptor serum stimulation-2 (ST2) (2). In 2006, the identity between IL-33 and NF-HEV and its role as a chromatin-related nuclear factor was further confirmed (3). IL-33 is produced by various cell types such as endothelial cells, epithelial cells, macrophages, fibroblasts, adipose progenitor cells, and dendritic cells. Under conditions of cell damage, necrosis, necroptosis, stress, and virus infection, it is released as a pro-inflammatory factor and activates different types of immune cells (4–7). The role of IL-33 in type 2 immune diseases has been extensively studied in allergic reactions, asthma, and parasitic infections (6). However, it is well-known that HEV is involved in the activation and mobilization of lymphocytes, indicating that IL-33 may also be involved in chronic inflammation (8, 9). Rheumatic diseases are chronic inflammatory disorders in which the immune system attacks itself and organs of the body. As an incurable condition so far, it brings a heavy burden to individuals and society (10). A growing number of studies have demonstrated a critical role of the IL-33/ST2 axis in rheumatic diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), primary Sjögren's syndrome (pSS), systemic sclerosis (SSc), psoriatic arthritis (PsA), gout, IgG4-related diseases, and ankylosing spondylitis (AS), indicating a promising potential for IL-33/ST2-targeting therapy in rheumatic disease (10).

BIOLOGY OF IL-33

The Distribution and Function of IL-33

Unlike some cytokines, which have classical secretion patterns, IL-33 is normally localized in the nucleus (11, 12). Although the localization of IL-33 in the cytoplasm has been reported in the literature, the results were not obtained under normal conditions. This ectopic expression was observed in murine cell line NIH3T3 that expressed tetracysteine-labeled human IL-33 by genetic engineering. Because the cysteine residues can change the folding of IL-33 and the fluorescence staining has not been tested by knockout, the results of cytoplasmic localization need to be treated with caution (13). The N-terminal domain of IL-33 shows evolutionary conservation and is closely related to the nuclear location of IL-33. The N-terminal domain of IL-33 was initially thought to contain homologous domain-like structures bound to deoxyribonucleic acid (DNA), but this has not been confirmed. In fact, IL-33 binds to DNA via protein–protein interactions. Through the tight hairpin structure of the chromatin-binding protein, it is combined with the acid pocket formed by histone 2A (H2A) and histone 2B (H2B) (1, 14). Although IL-33 is located in the nucleus, it does not appear to regulate the expression of genes. The nuclear localization of IL-33 seems to regulate the activity of IL-33 as a cytokine (15, 16). IL-33 is released outside of the cell and has a variety of immunological effects. Initially, researchers believed that the full-length IL-33 should be processed to be biologically active, and in the next few years, it was considered to be activated by caspase-1 and inflammasome, similar to IL-1β and IL-18. However, in 2009, Girard et al. reported that full-length IL-33 could interact with ST2 and activate nuclear factor-kappa B (NF-κB) activity to induce cytokine production (17, 18). Meanwhile, further studies also found the inflammatory protease hydrolysis site of IL-33. An 18- to 21-kilodalton (kDa) mature form can be produced when IL-33 is cleaved, with its biological activity level increased by 10–30 times (17, 19, 20). Although inflammatory proteases are able to convert IL-33 into a more active mature form, they may also result in the inactivation of IL-33 through protein degradation. This degradation has been observed in chymotrypsin. In addition, the endogenous caspase-3 can cleave at the DGVDG site in the C-terminal IL-1-like domain of IL-33 to inactivate IL-33. This structure is specific to IL-33, indicating that IL-33 is strictly regulated in the process of apoptosis (21). There was evidence showing that recombinant caspase-3 and caspase-7 could cleave IL-33 *in vitro*. Caspase-1 had no direct effect on IL-33 but could inactivate IL-33 by activating caspase-7 (22). In addition, when IL-33 was released into the extracellular microenvironment, it was quickly inactivated by the formation of two disulfide bonds. The oxidation of cysteine residues resulted in conformational changes and subsequent reduction in binding affinity to ST2. This regulation mechanism occurred much faster than protein degradation (23). Therefore, after a 2-h exposure to allergens, no biologically active IL-33 could be detected in the alveolar lavage fluid (23). Furthermore, IL-33 was found to accumulate in several models within a few hours after release and was not detectable after 6 h (24–26). These also reflect that IL-33 is a short-acting protein, and its biological role *in vivo* is precisely regulated.

In both physical and pathological inflammatory conditions, the main cellular sources of IL-33 are not CD45+ hematopoietic cells. Endothelial cells, epithelial cells, fibroblasts, and myofibroblasts in humans and mice were demonstrated to be the main cells expressing IL-33 (27). In addition to the epithelial barrier tissue and lymphatic organs, IL-33 was abundantly expressed in the brain and eyes of mice and weakly expressed in visceral smooth muscle cells of the human gastrointestinal tract and urogenital tract. Although several studies suggested that CD45+ hematopoietic cells may be a source of IL-33 even a major source, stronger evidence is needed (28–34). In an IL-33 luciferin reporter mouse model, no IL-33 was detected in CD45+ hematopoietic cells (including macrophages, dendritic cells, T cells, B cells, eosinophil, and neutrophil) in the lung of the mice with allergic pneumonia (35). It cannot be ruled out that certain leukocyte subsets may produce low levels of functional IL-33, but more experiments are still needed to verify it.

IL-33 Signaling Pathway

The receptor ST2, also known as DER4, Fit-1, or T1, is one of the co-receptors of IL-33 and is mainly encoded by the IL-1RL1 gene. Before the discovery of IL-33, ST2 was considered as an orphan receptor, and now IL-33 is still the only ligand of ST2 (36, 37). Three isoforms of ST2 have been identified in humans, all of which are produced by alternative splicing: transmembrane receptor type (ST2L), soluble form (sST2), and variant ST2 (ST2V) (38–40). The soluble form acts as a decoy receptor to antagonize IL-33. When IL-33 binds to the transmembrane ST2 receptor, the membrane-anchored ST2 will recruit IL-1 receptor accessory protein (IL-1RAcP) to form a dimer, resulting in the dimerization of its intracellular domain. The adaptor protein myeloid differentiation protein 88 (MyD88) is recruited through the dimerization of Toll/IL-1 receptor (TIR) to activate downstream kinases. IL-1R-associated kinase 1 (IRAK1) and IL-1R-associated kinase 1 (IRAK4) and TNF receptor-associated factor 6 (TRAF6) are then activated, which ultimately leads to the activation of mitogen-activated protein kinases (MAPKs) and NF-κB transcription factor (41–44). This signal pathway is very similar to that of IL-1β and IL-18. Unlike IL-1RAcP, IL-33 stimulation induces a binding of ST2 with single immunoglobulin domain IL-1R-related molecule (SIGIRR) to form a complex, which can inhibit the formation of intracellular dimers and activate the ubiquitin–proteasome system (45) (**Figure 1**).

Effects of IL-33 on Tissue Cells and Innate Immune Cells

Many types of cells express IL-33 receptors, including epithelial cells, endothelial cells, fibroblasts, and osteoblasts. IL-33 activates extracellular signal-regulated kinase (ERK) and p38 MAPK signaling pathways in primary human lung epithelial and endothelial cells to produce IL-8, which are associated with chronic airway inflammation (46). In addition, the IL-33

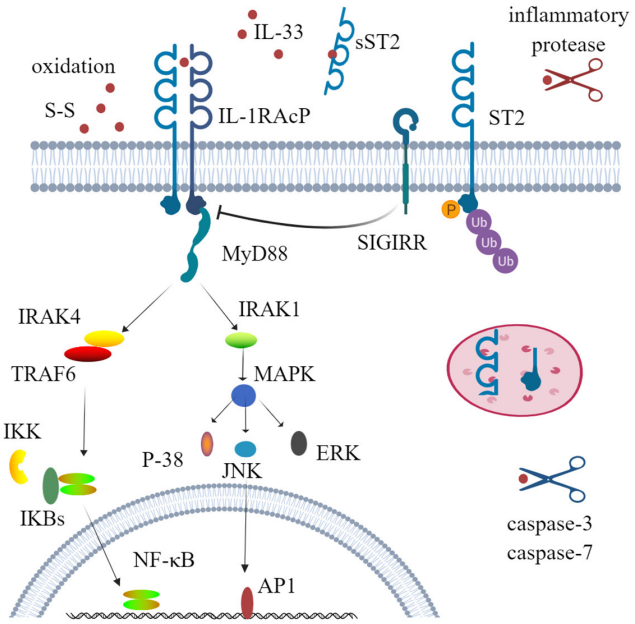

FIGURE 1 | Physiological function and activity regulation of interleukin-33 (IL-33). IL-33 firstly binds to its transmembrane receptor serum stimulation-2 (ST2) to induce conformational changes; then ST2 interacts with IL-1RAcP and recruits the downstream adaptor MyD88, IRAK1, IRAK4, and TRAF6 via Toll/IL-1 receptor domains; and ultimately activates nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (ERK, p38, and JNK). This signal pathway can also be inhibited by phosphorylation and ubiquitylation of ST2 and SIGIRR, which disrupt ST2 and IL-1RAcP dimerization. Several mechanisms are involved to regulate the activity of IL-33. Processing by inflammatory proteases can greatly increase (up to 30-fold) cytokine activity, while caspase-3 and caspase-7 lead to cytokine inactivity. Decoy receptor soluble ST2 (sST2) and rapid oxidation of IL-33 are also crucial mechanisms to limit the activity of IL-33. SIGIRR, single immunoglobulin domain IL-1 receptor-related molecule; IRAK, IL-1R-associated kinase; TRAF6, TNF receptor-associated factor 6; ERK, extracellular signal-regulated kinase.

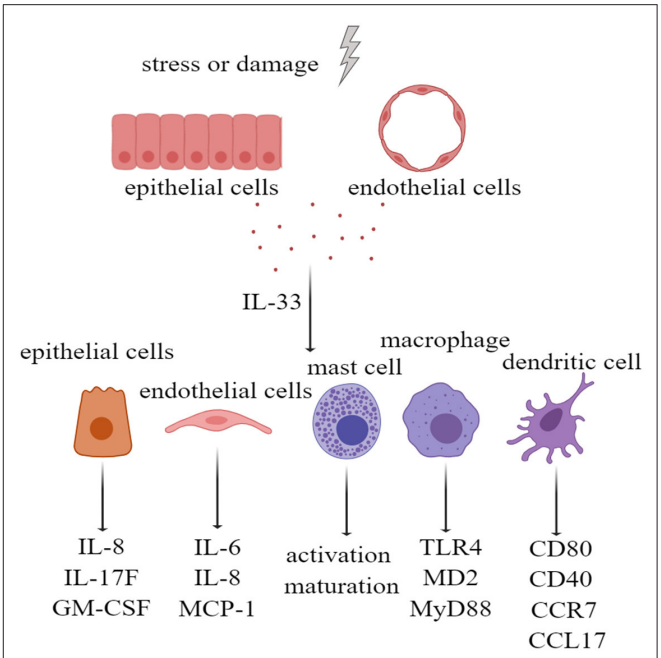

FIGURE 2 | Interleukin-33 (IL-33), as alarmin, activates tissue cells and a variety of innate immune cells. Extracellular IL-33, acting on bronchial epithelial cells, led to upregulation of IL-8, IL-17F, and GM-CSF. IL-33 also upregulated IL-6, IL-8, and MCP-1 by stimulating pulmonary endothelial cells. IL-33 not only stimulated the activation and maturation of mast cells, and upregulation of TLR4, MD2, and MyD88 in macrophages, but also upregulated CD80, CD40, CCR7, and CCL17 in dendritic cells. GM-CSF, granulocyte-macrophage colony stimulating factor; MCP-1, monocyte chemoattractant protein-1; TLR-4, Toll-like receptor 4; MD2, myeloid differentiation protein-2; MyD88, myeloid differential protein-88; CCR7, cxc chemokine receptor 7; CCL17, chemokine (C-C motif) ligand 17.

receptor ST2 is expressed on a variety of innate immune cells. IL-33/ST2 activation in mast cells not only promotes the activation and maturation of mast cells but also enhances Th17 response during airway inflammation (47, 48). In macrophages, IL-33/ST2 signaling enhances their activation by upregulating Toll-like receptor 4 (TLR4), myeloid differentiation protein-2 (MD2), and MyD88 (49). In the dendritic cells, the administration of IL-33 not only increases the levels of CD80, CD40, and C-C motif chemokine receptor 7 (CCR7) but also increases the production of IL-5, C-C motif chemokine ligand 17 (CCL17), and tumor necrosis factor alpha (TNF-α) (50). Therefore, IL-33, as an alarmin and damage-associated molecular pattern (DAMP) molecule, can activate tissue cells and innate immune cells; upregulate costimulatory molecules, adhesion molecules, and chemokines; and initiate and maintain innate immunity (**Figure 2**).

IL-33 Indirectly Promotes the Production of IFN-γ

Interferon-gamma (IFN-γ) plays an important role in the development of rheumatic immune disease. IFN-γ can activate macrophages or other immune cells to aggravate tissue damage and can promote the ectopic expression of MHC class II antigens on tissue cells, which may contribute to the presentation of autoantigen. Several studies have proven that IL-33, in the presence of IL-12, can increase the secretion of IFN-γ by NK cells, natural killer T cells (NKT cells), ILC1 cells, and Th1 cells (51–54). Therefore, in the progression of rheumatic disease, IL-33 may increase the production of IFN-γ and may amplify the immune effects (**Figure 3**). In addition, IL-33 is also thought to increase antibody levels in the immune response.

IL-33 Promotes Tissue Repair and Fibrosis

Although IL-33 is an alarmin and is involved in inflammatory processes, there is evidence that IL-33 plays a role in wound healing and fibrosis. IL-33/ST2 signal enables M2 macrophages to promote the closure of damaged epidermis and angiogenesis, etc. In addition, M2 macrophages are also involved in tissue remodeling and fibrosis (55). In addition, IL-33 can act on eosinophils, mast cells, and ILC2 to increase their production of IL-13 and IL-5, which are closely related to fibrosis (56). More importantly, IL-33 can increase the number of ST2+ regulatory T cell (Treg), a pivotal type of cells in fibrogenesis and immunosuppression (57). IL-33 is also believed to affect the

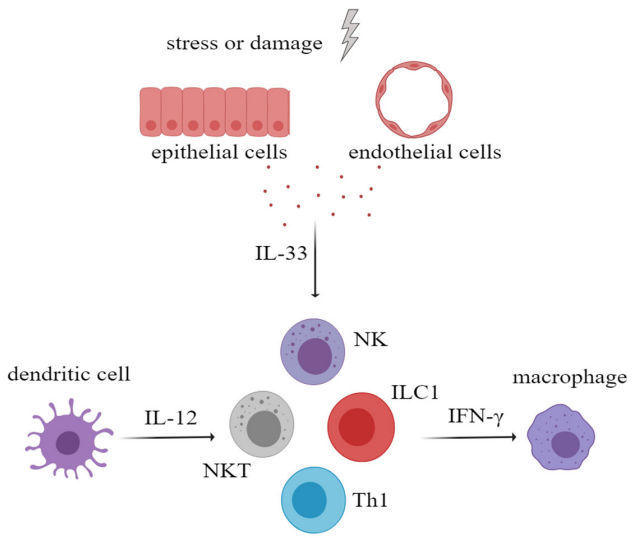

FIGURE 3 | After activation of innate immune cells, especially myeloid dendritic cells, large amounts of interleukin-12 (IL-12) were secreted. IL-33 and IL-12 had a synergistic effect in stimulating NK cells, NKT cells, ILC1, and Th1 to secrete more IFN-γ, which will aggravate tissue damage. NK cells, natural killer cells; NKT cell, natural killer T cell; ILC, innate lymphoid cell.

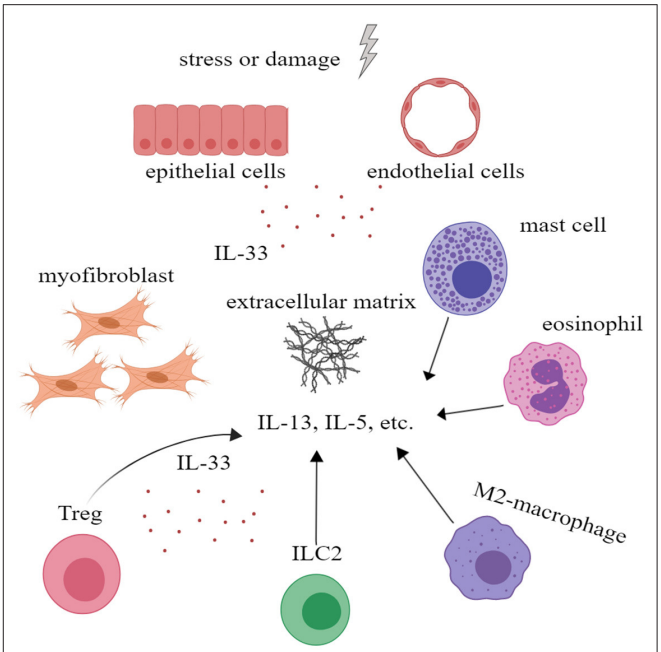

FIGURE 4 | Interleukin-33 (IL-33) could promote tissue repair and fibrosis. IL-33 could promote the proliferation of Treg to further alleviate inflammation. IL-33 could also promote extracellular matrix deposition and tissue fibrosis by acting on mast cells, eosinophil, M2 macrophage, ILC2 cells, and Treg to increase the secretion of IL-5, IL-13, and other cytokines. ILC, innate lymphoid cell; Treg, regulatory T cell.

activity of matrix metalloproteinases and promote the deposition of extracellular matrix (56). All these indicate that IL-33 plays an important role in tissue repair and fibrosis (**Figure 4**).

IL-33/ST2 AXIS IN RHEUMATIC DISEASES

Rheumatic diseases are immune-mediated chronic inflammatory syndromes, which are characterized by the hyperactivity of effector Th1 cells and Th17 cells, dysfunction of Tregs, activation of autoreactive B cells, and production of autoantibodies (58). A growing number of studies have found that the level of IL-33 is associated with the severity of rheumatic disease, indicating that IL-33 and ST2 may be potential targets for predicting the development of disease and improving the clinical outcomes (59, 60). Next, we will discuss the role of the IL-33/ST2 axis in several common rheumatic diseases (**Table 1**, **Figure 5**).

Systemic Lupus Erythematosus

SLE is a chronic connective tissue disease of unknown etiology with multiple systemic involvements. The male-to-female ratio is about 1:9, and it is a major cause of death in young women with chronic inflammatory diseases (97). The prevalence of SLE is (30.13–70.41)/100,000 in China. The level of serum IL-33 was reported to be significantly higher in patients with SLE than that in healthy subjects and was positively correlated with erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IgA, and Sjögren's syndrome antigen B (SSB) antibody levels (61). On the other hand, the serum soluble ST2 (sST2) level in patients was also significantly increased and was positively correlated with the level of anti-double-stranded DNA (dsDNA) antibodies and the disease activity index, while it was negatively correlated with the complement C3 (62). In addition, Guo et al. suggested that IL-33 and other pro-inflammatory cytokines might be involved in innate lymphoid cell disorder with a higher frequency of ILC1 in the peripheral blood of SLE patients (63). However, there were also other studies showing reduced or similar levels of serum IL-33 in SLE patients compared with healthy controls (62). This discrepancy is probably due to the detection efficacy of the enzyme-linked immunosorbent assay (ELISA) kit or the multiple roles of IL-33 plays in the disease (98). To further explore its effect, Li et al. found that the administration of neutralizing antibodies against IL-33 could reduce the mortality, serum anti-dsDNA level, and immune complex deposition in MRL/lpr lupus mice. The protective effect was associated with the increase of regulatory T cells and myeloid-derived suppressor cells and the reduction of Th17 cells and pro-inflammatory factors (64). These studies also indirectly supported the finding that two polymorphisms of the IL-33 gene (rs1929992-G and rs1891385-C) were associated with increased susceptibility to SLE (99–101). However, further studies are required to further explore the corresponding mechanism.

Rheumatoid Arthritis

RA is an autoimmune disease with erosive arthritis as the main manifestation and synovitis as the pathological basis (102). The male-to-female ratio is 1:4. In severe cases, patients with uncontrolled active RA may develop joint deformities and disabilities. The prevalence of RA in China is 0.28–0.41%.

TABLE 1 | Expression and mechanisms of IL-33/ST2 in rheumatic diseases.

Disease	Role of IL-33/ST2 in disease pathogenesis	Referencess
SLE	IL-33 and sST2 levels were increased in the serum of SLE patients. IL-33 neutralization had a protective effect in MRL/lpr mice, which was associated with the increase of regulatory T cells and myeloid-derived suppressor cells and the reduction of Th17 cells and pro-inflammatory factors. IL-33 might be involved in innate lymphoid cell disorder in the peripheral blood of SLE patients.	(61–64)
RA	IL-33 levels were related to the severity and activity of RA. IL-33 enhanced TNF-α-dependent effects in synovial fibroblasts. $ST2^{-/-}$ and ST2 neutralization in the CIA model alleviated arthritis symptoms, while administration of IL-33 exacerbated. IL-33 stimulates mast cells to produce pro-inflammatory factors. IL-33 stimulated macrophages and synovial cells to produce chemokines, which recruited neutrophils.	(65–71)
pSS	IL-33 and sST2 levels were increased in the serum of pSS patients. IL-33 promoted the release of IFN-γ in NK and NKT cells when combined with IL-12 and/or IL-23.	(72–74)
SSc	IL-33 and sST2 levels were increased in the serum of SSc patients. IL-33 levels were correlated with skin lesions, degree of sclerosis. IL-33 polarized M2 macrophages to produce TGF-β1 and IL-13, induced ILC2 proliferation, increased eosinophils and the level of IL-13, and induced Treg dysfunction.	(75–79)
PsA	IL-33 levels were increased in the serum of patients. IL-33 increased the gene expression of the pro-osteoclastogenic factor associated with bone damage.	(80, 81)
Gout	IL-33 levels were increased in joint synovial fluid. Exogenous administration of IL-33 aggravated the production of ROS and recruitment of neutrophils, while knocking out ST2 alleviated the oxidative stress and neutrophils recruitment. IL-33 stimulated macrophages to produce CXCL1, CCL 3, and IL-1β. IL-33 recruited bone marrow-derived suppressor cells and reduced the production of IL-1β.	(82–84)
IgG4-RD	IL-33 levels were increased in the serum of patients. Prednisolone treatment decreased the serum concentration of IL-33. IL-33 activated the Th2 immune response and promoted tissue fibrosis.	(85–87)
AS	Serum IL-33 levels were elevated in the patients with AS. IL-33 enhanced the production of TNF-α and IL-6 in peripheral blood mononuclear cells and induced neutrophil migration. IL-33 was used as a predictor of the therapeutic effect of infliximab in the treatment of AS.	(88–91)
IIM	Serum sST2 levels were elevated and correlated with CRP, CK, and LDH.	(92, 93)
AOSD	Serum IL-33 and sST2 levels were elevated and correlated with ferritin levels.	(94)
BD	Serum and skin tissue of IL-33 and sST2 levels were elevated in patients.	(95, 96)

SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; CIA, collagen-induced arthritis; pSS, primary Sjögren's syndrome; NK, natural killer cells; NKT, natural killer T cells; SSc, systemic sclerosis; PsA, psoriatic arthritis; ROS, reactive oxygen species; IgG4-RD, IgG4-related disease; AS, ankylosing spondylitis; IIM, idiopathic inflammatory myopathies; CRP, C-reactive protein; CK, creatine kinase; LDH, lactate dehydrogenase; AOSD, adult-onset Still's disease; BD, Behcet's disease.

Immunopathology showed that the differentiation of Th1 lymphocytes increased and that the number of Treg decreased in RA patients (103). Elevated IL-33 levels were found in both serum and local joint synovial fluid in patients with RA (65, 66, 103). Serum sST2 levels were also elevated (68). Moreover, IL-33 levels were associated with RA severity parameters, such as rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP) antibodies, IL-6, ESR, lung involvement, and bone erosion (68, 104–106). IL-33 was also expressed in synovial fibroblasts (69). Inflammatory factors (such as TNF-α) could stimulate synovial fibroblasts to produce IL-33; and IL-33 not only upregulated matrix metalloproteinase-3 (MMP-3), IL-8, and IL-6 but also upregulated B-cell lymphoma-2 (Bcl-2) to inhibit apoptosis and promote proliferation (107). Furthermore, the level of serum IL-33 also had a certain significance for the prediction of patient's response to the biological agents. For example, in patients with poor response to tumor necrosis factor inhibitor (TNFi), serum and synovial fluid IL-33 levels continued to rise (70). In the mouse model, IL-33 mRNA levels increased in the early stages of collagen-induced arthritis (CIA) (65). With the use of ST2 knockout mice and ST2 neutralizing antibodies in the CIA model, suppression of ST2 signaling alleviated arthritis symptoms and reduced levels of IL-17, TNF-α, and IFN-γ (65, 69). In contrast, the administration of IL-33 resulted in the exacerbation of arthritis (108). Interestingly, this effect was absent in mice lacking mast cells. Further studies showed that mast cells expressed high levels of ST2, which responded to IL-33 by producing various pro-inflammatory factors such as monocyte chemoattractant protein-1 (MCP-1), IL-6, and IL-1β (69). In addition, IL-33 could also recruit neutrophils by stimulating macrophages and synovial cells to release chemokines (such as CXCL1 and CCL3) (71). Although most studies support the deleterious effect of the IL-33/ST2 axis in the pathogenesis of RA, there are still some studies with opposite results. For example, repeated administration of IL-33 in the early and late stages of CIA mice models could relieve symptoms of arthritis. The protective mechanism might involve the regulation of immunity and the proliferation of Tregs (109). Despite the controversy, research from the gene polymorphism suggests that downregulating the expression of IL-33 shows resistance to disease. Further studies are needed to explore the specific role and molecular mechanisms of IL-33 in RA.

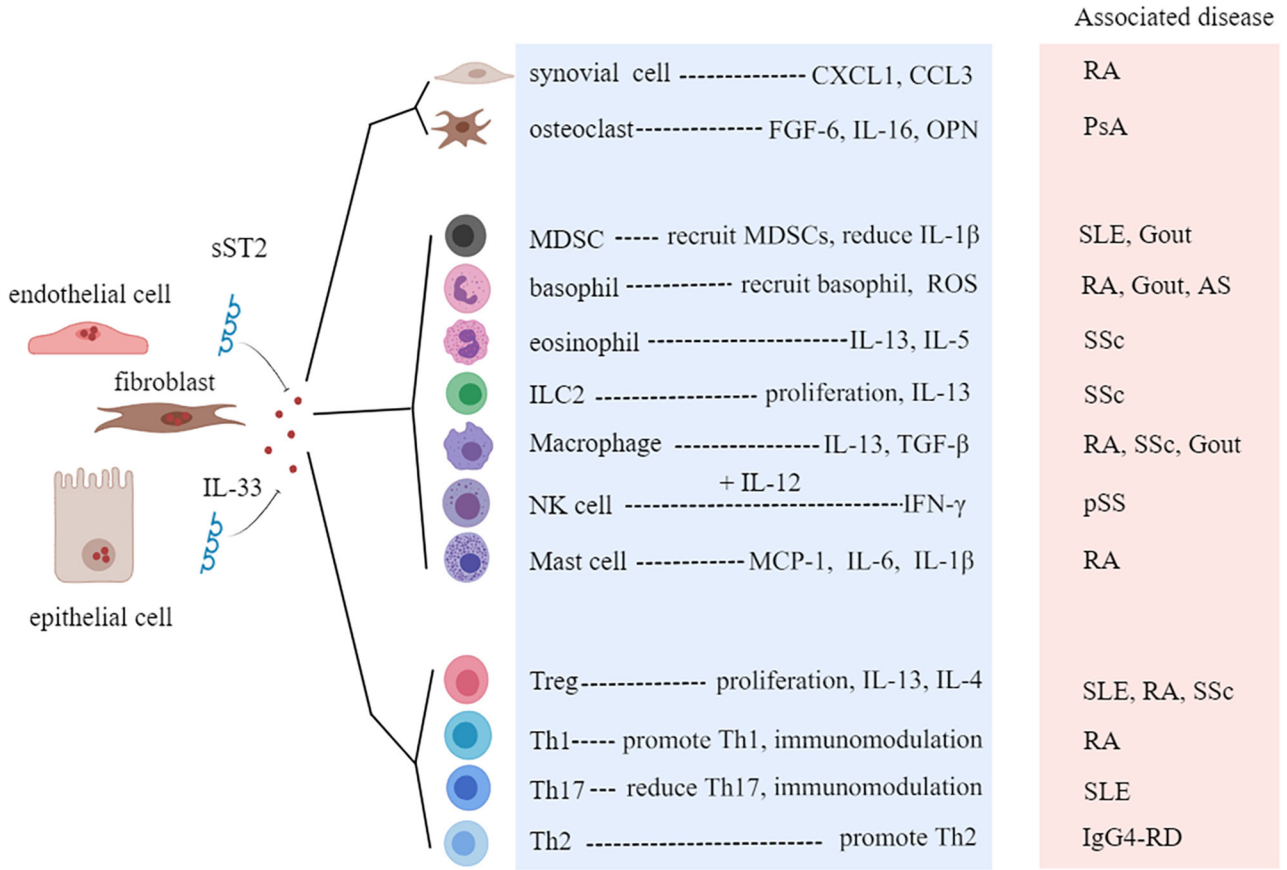

FIGURE 5 | Roles of interleukin-33 (IL-33) in rheumatic diseases. sST2, soluble ST2; CXCL1, C-X-C motif chemokine ligand 1; FGF-6, fibroblast growth factor 6; OPN, osteopontin; MDSC, myeloid-derived suppressor cell; ROS, reactive oxygen species; ILC, innate lymphoid cell; Treg, regulatory T cell; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; pSS, primary Sjögren's syndrome; SSc, systemic sclerosis; PsA, psoriatic arthritis; IgG4-RD, IgG4-related disease; AS, ankylosing spondylitis.

Primary Sjögren's Syndrome

pSS is a chronic systemic rheumatic disease mainly involving salivary and lacrimal glands. The male-to-female ratio is 1:(9–20) (110). The prevalence of pSS in China is 0.28–0.41%. Multiple studies have described the pathogenic role of the IL-33/ST2 axis in patients with pSS (72, 73, 111–113). Compared with those in the control group, serum IL-33 and sST levels were elevated in pSS patients. Although serum IL-33 levels and EULAR Sjögren's syndrome disease activity index (ESSDAI) or lymphocyte infiltration were not correlated, serum sST2 levels were significantly correlated with ESSDAI, disease duration, and thrombocytopenia (72, 73). Immunohistochemical staining showed that IL-33 and its receptors (ST2 and IL-1RAcP) were expressed in the salivary glands. The expression of IL-33 in patients with pSS showed a dynamic pattern: IL-33 was significantly increased in salivary glands with Chisholm scores of 2 and 3 but was expressed at a lower level in salivary glands with Chisholm scores of 1 and 4. Its receptors (ST2 and IL-1RAcP) were expressed in a similar pattern (73). In addition, a recent study showed that the levels of IL-33 in the tears of patients with pSS were also significantly increased. Furthermore, IL-33 levels were correlated with the degree of ocular involvement and levels of IL-4 and IL-5 in tears (74). However, IL-33 alone did not lead to the release of pro-inflammatory factors. But when combined with IL-12 and/or IL-23, it promoted the release of IFN-γ by up to 10 times in NK and NKT cells. Moreover, TNF-α, IL-1β, and IFN-γ in the inflammatory environment could further increase the activation of IL-33, forming positive feedback (60). Therefore, the targeting IL-33/ST2 axis may be a promising treatment option for pSS.

Systemic Sclerosis

SSc is a rheumatic disease with unknown etiology characterized by the deposition of the extracellular matrix and diffuse skin thickening (114). The male-to-female ratio is 1:(3–7), and the lence rate in China is about 0.026%. It was reported that the serum IL-33 level in patients with early SSc was higher than that of healthy controls and patients with advanced SSc. This might be due to the activation of endothelial cells in the early stages of the disease, and elevated IL-33 level was positively correlated

with skin lesions, degree of sclerosis, and degree of pulmonary fibrosis (75, 76). In skin biopsies of healthy subjects, ST2 was only expressed in fibroblasts and endothelial cells at a low level, while IL-33 was constitutively expressed in keratinocytes and endothelial cells (77). However, in the early stage of SSc, ST2 was highly expressed in endothelial cells, macrophages, T cells, B cells, and myofibroblasts of the affected organs, while the expression of IL-33 in tissues was not significantly increased until the late stage of SSc (77). In fact, the IL-33/ST2 axis could polarize M2 macrophages to promote the production of TGF-β1 and IL-13 and could also induce the proliferation of ILC2 to promote the accumulation of eosinophil granulocyte and expression of IL-13. Moreover, *in vitro* experiments have shown that IL-33 could induce the differentiation of Tregs into Th2-like cells, resulting in the production of IL-4 and IL-13 and local Treg dysfunction (77–79). The study in our laboratory also found that IL-33 could directly promote the proliferation of primary human skin fibroblasts and their expression of collagen. The administration of ST2 neutralizing antibody was able to effectively alleviate bleomycin-induced skin fibrosis in mice. Moreover, the polymorphism of IL-33 gene rs7044343 is associated with SSc-associated dyspnea in the Chinese population and SSc susceptibility in the Turkish population (115, 116). However, further research is needed to determine the therapeutic effect of IL-33/ST2 targeting therapy in human SSc.

Psoriatic Arthritis

PsA is a chronic, inflammatory, musculoskeletal disease affecting the skin, peripheral joint, spine, nails, and entheses (117). It was reported that up to 30% of patients with psoriasis might develop PsA (118). Several studies showed that IL-33 not only played a role in the development of psoriasis but also participated in the progress of PsA (119). One study detected elevated serum IL-33 in patients with PsA, but there was no correlation with osteoclastogenesis-related cytokines and PSA joint activity index (PSAJAI) (80). In another study, however, IL-33 was not detected in serum and synovial fluid from PsA patients but only in endothelial cells of the synovium and synovial fibroblast (120). Another study focused on the effects of skin inflammation on bone damage. IL-33 together with IL-17 increased the gene expression of the pro-osteoclastogenic factor, such as fibroblast growth factor (FGF-6), IL-16, and osteopontin (OPN). Moreover, IL-33, together with OPN, IL-17, and TNF-α, could also induce the release of bone contributing factor receptor activator of NF-κB ligand (RANKL) in the skin, thus inducing the differentiation of osteoclast precursor (OCP) into monocytes (81). IL-33 was also expressed in the synovium in a mouse model of PsA. But there was no difference between IL-33$^{-/-}$ and wild-type (WT) mice in frequencies of Treg, Th1, and Th17 cells in this model (121). In conclusion, the present studies suggest that IL-33 is involved in the development of human PsA, while studies in mouse models are limited. Further studies are needed to obtain more evidence.

Gout

Gout is an inflammatory disease characterized by the deposition of uric acid crystals in the joints (122). The male-to-female ratio is 15:1, and its incidence in China is 1% to 3%. It was reported that higher levels of IL-33 and neutrophil counts were detected in joint synovial fluid in patients with gout than those in osteoarthritis (82). Direct injection of uric acid crystals into the articular cavity could induce acute attacks of gout in mice. In this mice model, exogenous administration of IL-33 could aggravate the production of reactive oxygen species, recruitment of neutrophils, and hyperalgesia. Correspondingly, knocking out ST2 could significantly alleviate oxidative stress and reduce neutrophils' recruitment into the ankle joint. It was also found that macrophages in gout could produce IL-33 and increase CXCL1, CCL 3, and IL-1β through an autocrine pattern. These results supported the pathogenic role of the IL-33/ST2 axis in gout (83). Paradoxically, IL-33 was also believed to alleviate the mouse peritonitis model induced by uric acid crystals and to reduce neutrophil counts as well as the production of IL-1β and IL-6 (84). The mechanism was associated with the recruitment of bone marrow-derived suppressor cells by IL-33, which reduced the production of IL-1β in the peritoneal cavity (84). These results also precisely reflect the distinct action patterns of IL-33 in different sites and under different pathological conditions.

IgG4-Related Disease

The IgG4-related disease (IgG4-RD) is an idiopathic, fibroinflammatory disease characterized by elevated serum IgG4 levels, tumefaction, and tissue infiltration by IgG4-positive plasma cells (123). The ratio of male to female is ∼(2–3):1. The current incidence in China is unknown. However, with the improvement in disease cognition and detection, the number of patients is gradually increasing. Furukawa et al. found that IL-33 could act as an inducer of Th2 response in IgG4-RD (85). Further studies found that in patients with IgG4-related autoimmune pancreatitis, plasmacytoid dendritic cells could produce IL-33 and interferon alpha (IFN-α), which were closely related to the fibrosis of the disease. The authors further validated these results in a mice model and demonstrated that depletion of plasmacytoid dendritic cells and blockade of signaling pathways related to type 1 interferon and IL-33 could prevent chronic fibrosis (124). Treatment with prednisolone was able to improve the swelling of the pancreas with a significant reduction of serum IFN-α and IL-33, but the serum IgG, IgG4, and IgE concentrations only slightly decreased. This suggested that the IFN-α/IL-33 axis may be a better biomarker reflecting the disease activity of IgG4-RD compared with serum levels of IgG, IgG4, and IgE (86, 125, 126). There is no correlation between serum IL-33 and serum IgG4 or IgG4:IgG ratio (126). Another study on IgG4-related sialadenitis demonstrated that TLR7-positive M2 macrophage was able to produce high levels of IL-33 *in vitro*, which activated the Th2 immune response and promoted tissue fibrosis in IgG4-RD (87). In conclusion, IL-33 plays an important role in the development of IgG4-related diseases as an important inducer of type 2 immunity and an important pro-fibrogenic factor. However, the specific mechanism is still unclear.

Ankylosing Spondylitis

AS is a chronic, inflammatory rheumatic disease affecting the spine and sacroiliac joints. The main clinical features are inflammatory back pain, bone erosion, and syndesmophyte

formation (127). In China, the prevalence of AS is 0.25∼0.5%, and the ratio of male-to-female is about 4–1. Due to the lack of suitable animal models, all studies of IL-33 in AS have been conducted in humans. Serum IL-33 levels were reported to be elevated in patients with AS, especially in patients with active AS (88). In addition, IL-33 enhanced the production of TNF-α and IL-6 in peripheral blood mononuclear cells (PBMCs) and induced neutrophil migration when the dose of IL-33 exceeded 10 ng/ml (89). Another study explored the relationship between IL-33 gene polymorphisms and disease susceptibility and found that AS patients carrying the IL-33 rs16924159 AA genotype had higher disease activity and a worse response to anti-TNF therapy (90). But overall, IL-33 could be still used as a predictor of the therapeutic effect of infliximab in the treatment of AS (91). These results suggest that IL-33 is involved in the pathogenesis of AS and is a potential therapeutic target, but more studies are still needed.

Other Rheumatic Diseases

IL-33 also played a role in other rheumatic diseases such as idiopathic inflammatory myopathies (IIM), adult-onset Still's disease (AOSD), and Behcet's disease (BD).

IIM is a chronic rheumatic disease, which can lead to skin and internal organ involvement. IIM includes dermatomyositis (DM) and polymyositis (PM). It was reported that serum sST2 levels were significantly elevated in DM and PM patients and decreased after treatment. In addition, serum sST2 levels were correlated with CRP, creatine kinase (CK), and lactate dehydrogenase (LDH) (92). In another study, serum IL-33 could not be detected in the majority of IIM patients, but serum sST2 levels were elevated and even much higher in patients with anti-signal recognition particle (anti-SRP) antibodies (93). Considering the abnormal expression of sST2 and the short detection time window of IL-33, it can be speculated that IL-33 may be involved in the pathogenesis of IIM.

AOSD is a rare systemic inflammatory disorder, which is characterized by high spiking fever, an evanescent rash, polyarthralgia, arthritis, and hepatosplenomegaly. It was reported that serum levels of IL-33 and sST2 were elevated in patients with active AOSD; and serum IL-33 levels correlated with systemic score, ESR, ferritin levels, and aspartate transaminase levels, while serum soluble ST2 levels correlated only with ferritin levels (94). These results indicated that the IL-33/ST2 signaling pathway may play a role in the pathogenesis of AOSD.

BD is a multisystem inflammatory disease, characterized by recurrent oral ulceration, skin lesions, genital ulcerations, and uveitis. Serum and skin tissue of IL-33 and sST2 levels were reported to be elevated in patients with BD, and sST2 is associated with ESR and CRP (95). Another study reported that in BD patients of Iran, the expression of IL-33 mRNA in PBMCs was much higher than in healthy controls, and rs1342326 T/G polymorphism of the IL-33 gene might contribute to the genetic susceptibility to BD (96). These results suggest that IL-33 may play an important role in the pathogenesis of BD.

DISCUSSION

In this review, we summarize the role of the IL-33/ST2 axis in rheumatic diseases by summarizing the evidence from clinical patients, mouse models, and *in vitro* cell culture. IL-33 is characterized as an alarmin, with ILC2, Th2, and Tregs as the main target cells in immune system. Because of the complexity and functional diversity of IL-33, it may play distinct roles in different stages of disease and different immune microenvironments. For example, administration of exogenous IL-33 with different treatment duration, different concentrations, or different stage of disease may result in different, even opposite, therapeutic effects. Nevertheless, previous studies have demonstrated a detrimental role of IL-33/ST2 axis in RA, scleroderma, SLE, psoriasis, and gout. The potential mechanisms may involve the immunomodulation, fibrogenesis, and tissue repair.

IL-33 is able to promote the polarization of macrophages, activate mast cells ILC2, and induce eosinophil activation. We and others have shown that some tissue cells including epithelial cells and fibroblasts also express IL-33 and its receptor ST2. IL-33 can also participate in the pathogenesis of rheumatic disease by interacting with ST2-expressing tissue cells, such as cardiomyocytes, oligodendrocytes, epithelial cells, and endothelial cells. The activation, dysfunction, and destruction of these cells are directly involved in the development of many rheumatic diseases. In general, IL-33/ST2 axis plays a detrimental role in both early and advanced stages of most rheumatic diseases. In the early stages of the disease, IL-33 can be released from damaged epithelial cells acting as an alarmin to activate other local tissue cells and recruit immune cells. In addition, IL-33 seems to interact with various cytokines such as IL-12 to produce more IFN-γ in the early inflammation environment. In contrast, during the advanced repair and fibrosis stages, IL-33 as an important pro-fibrogenic factor may play a protective role in maintaining the integrity of the barrier system. These characteristics determine the pleiotropy and complexity of IL-33.

CONCLUSION

In summary, the sources and targets of IL-33 involve a variety of cell types. Although great advances have been made in recent years, more evidence is needed to clarify the exact role of the IL-33/ST2 axis in rheumatic diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Effect of Clinical Typing on Serum Urate Targets of Benzbromarone in Chinese Gout Patients: A Prospective Cohort Study

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Introduction: Achieving a goal of serum urate levels in patients with gout is an important way to prevent gout and its complications while it remains difficult with a low targeting rate worldwidely. Currently, hyperuricemia classification has not been widely applied to the management of gout owing to insufficient clinical evidences. This study aimed to evaluate the effectiveness of achieving target urate based on hyperuricemia classification in Chinese patients with gout.

Methods: In this prospective study, patients with gout receiving urate lowering therapy with benzbromarone were assigned to two groups, a renal underexcretion and an unclassified type. The primary endpoint was the proportion of patients achieving the serum urate target (< 360 μmol/L) during the 12-week study. The frequency of acute gout attacks as well as physical and chemical indicators were secondary endpoints.

Results: Target serum urate level was achieved in 60.5% of underexcretors compared with 39.0% of patients of the unclassified type at week 12 ($P = 0.002$). Blood glucose and cholesterol levels were lower in the underexcretor group compared with the unclassified type group at the end of the trial, without significant different frequencies in gout flare during the study. In subgroup analysis, stratified by body mass index and estimated glomerular filtration rate, the proportion of patients with serum urate < 360 μmol/L was greater in the underexcretion compared with the unclassified type group.

Conclusions: The increased achievement of target serum urate in the underexcretion group supports the use of a clinical hyperuricemia typing treatment strategy for gout.

Keywords: gout, clinical typing, serum urate target, low-dose benzbromarone, urate-lowering therapy

INTRODUCTION

Gout is a disease with a metabolic basis primarily caused by reduced urate excretion (1). Gout leads to joint deformity, and is associated with kidney damage (2) (uremia), and induces or aggravates diabetes (3) and cardiovascular diseases (4) when hyperuricemia in patients with gout is inadequately treated. The goal of gout therapy is to lower serum urate (SU) below the threshold of supersaturation (< 360 μmol/L) to prevent any gouty attack by allowing the dissolution of existing monosodium urate (MSU) crystals (5), as well as to improve the heart and kidney complications (6, 7). However, the poor adherence to urate-lowering therapy (ULT) and clinical inertia induces a poor achievement of target SU (8–13), which puts forward new challenges to clinical diagnosis and treatment.

Hyperuricemia can be classified into the underexcretion type, the overproduction type, the combined type, and the extra-renal urate underexcretion type according to the fractional excretion of urate (FEUA) and 24-h urinary urate excretion (UUE) (14–16). Previous studies have indicated that the drug selection by hyperuricemia classification type can improve reduction in SU levels (17–19). But these findings have not been confirmed in an independent clinical trial.

Benzbromarone is a first-line urate-lowering drug in Asia, especially in China, while a second-line drug in European and American countries (20, 21). Given the large population treated with benzbromarone, it is particularly crucial to take hyperuricemia classification, for benzbromarone is not indicated for the patients caused by uric acid overproduction (22).

Thus far, only the 2006 European League Against Rheumatism (EULAR) evidence-based recommendations for gout suggest renal urate excretion should be determined to inform treatment in selected patients with gout, especially those with a family history of young onset gout, onset of gout under age 25, or with renal calculi (level IIb evidence) (23), while the 2020 American College of Rheumatology (ACR) Gout Clinical Practice Guidelines and 2020 recommendations from the French Society of Rheumatology for the management of gout advise against checking urine urate level for patients with gout due to the lack of evidence (24, 25).

Currently, the lack of clinical evidence hinders the application of hyperuricemia classification to the treatment of gout. The present study was designed to evaluate the effectiveness of treatment based on urate excretion classification in Chinese patients with gout with low-dose benzbromarone.

METHODS

Study Design and Patients

This single-center, controlled prospective clinical study was conducted in the dedicated Shandong Gout Clinic Medical Center at the Affiliated Hospital of Qingdao University. The clinical trial was conducted according to the principles from the Declaration of Helsinki, and approved by the Ethics Committee of the institution and registered in ChiCTR (#1900022981). Two hundred and forty Chinese male patients with gout were recruited between May 2019 and July 2020. All patients who participated in this study provided written informed consent. We enrolled patients who met the 2015 ACR/EULAR diagnostic criteria of primary gout (26) with 18–70 years of age. As the female patients were limited in our clinic and to minimize the influence of sex confounding, we only included males in this study. Key exclusion criteria were (1) SU < 420 μmol/L or SU > 600 μmol/L, (2) estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2, (3) presenting abnormally high levels of transaminases (> 2 times of the upper limit of normal, ULN), (4) suffering from renal calculus, polycystic kidney, rheumatoid arthritis, diabetes, or other serious complications, and (5) use of drugs affecting SU levels, such as losartan, fenofibrate, metformin, and hydrochlorothiazide. The reasons to exclude patients with gout with SU > 600 μmol/L are considerations of heavy burden to kidney with uricosuric agents and decreased efficacy of low-dose benzbromarone in the management of such high SU levels (27).

Assessments and Procedures

Before the start of the trial, all the participants were required to undertake a 2-week washout. During the period, the patients maintained a low purine diet and took no drugs. If there was a gout flare within the washout duration, the patients were prescribed colchicine or etoricoxib and restarted the washout process. Participants who underwent the classification test were required to conduct a 24-h urine collection 1 day prior to baseline visit. The total urinary volume in 24 h was also recorded.

Basic characteristics, including age, height, weight, disease duration of gout, frequency of attacks (per year), coexisting conditions, smoking history, alcohol drinking history, were collected. SU, blood urea nitrogen (BUN), serum creatinine (sCr), serum glucose (GLU), serum triglyceride (TG), serum cholesterol (TC), serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), 24-h urine urate (uUA), and creatinine (uCr) were measured using an automatic biochemical analyzer (TBA-40FR, Toshiba Company, Japan). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. eGFR was calculated using the Modification of Diet in Renal Disease formula (28). The FEUA and 24-h UUE were calculated by 24-h urine volume and 24-h uUA and uCr. FEUA= uUA/uCr × sCr/sUA × 100 % and UUE = uUA × 24-h urinary volume / [0.0061 × height (cm) + 0.0128 × weight (kg) − 0.1529) × 1.73 (mg/d/1.73m^2)] (29). The underexcretion type of hyperuricemia was defined as the FEUA $< 5.5\%$ and 24-h UUE ≤ 600 mg/day/1.73 m^2 (16, 29).

The same regime was implemented in both groups, i.e., 25 mg benzbromarone qd. and 1 g sodium bicarbonate tid. for 12 weeks. The patients were encouraged to drink water $\geq$ 2,000 mL daily. Follow-ups and biochemical measurements were requested at weeks 2, 4, 8, and 12 (**Supplementary Figure 1**). All patients were given low dose of colchicine (0.5 mg/day) during the trial to prevent acute attacks of gout. The patients were withdrawn

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Cr, Creatinine; GLU, Serum glucose; TC, Cholesterol; TG, Triglyceride; SU, Serum urate; BUN, blood urea nitrogen; MSU, Monosodium urate; FEUA, Fractional excretion of urate; UUE, Urinary urate excretion; ULN, Upper limit of normal; eGFR, Estimated glomerular filtration rate; BMI, Body mass index.

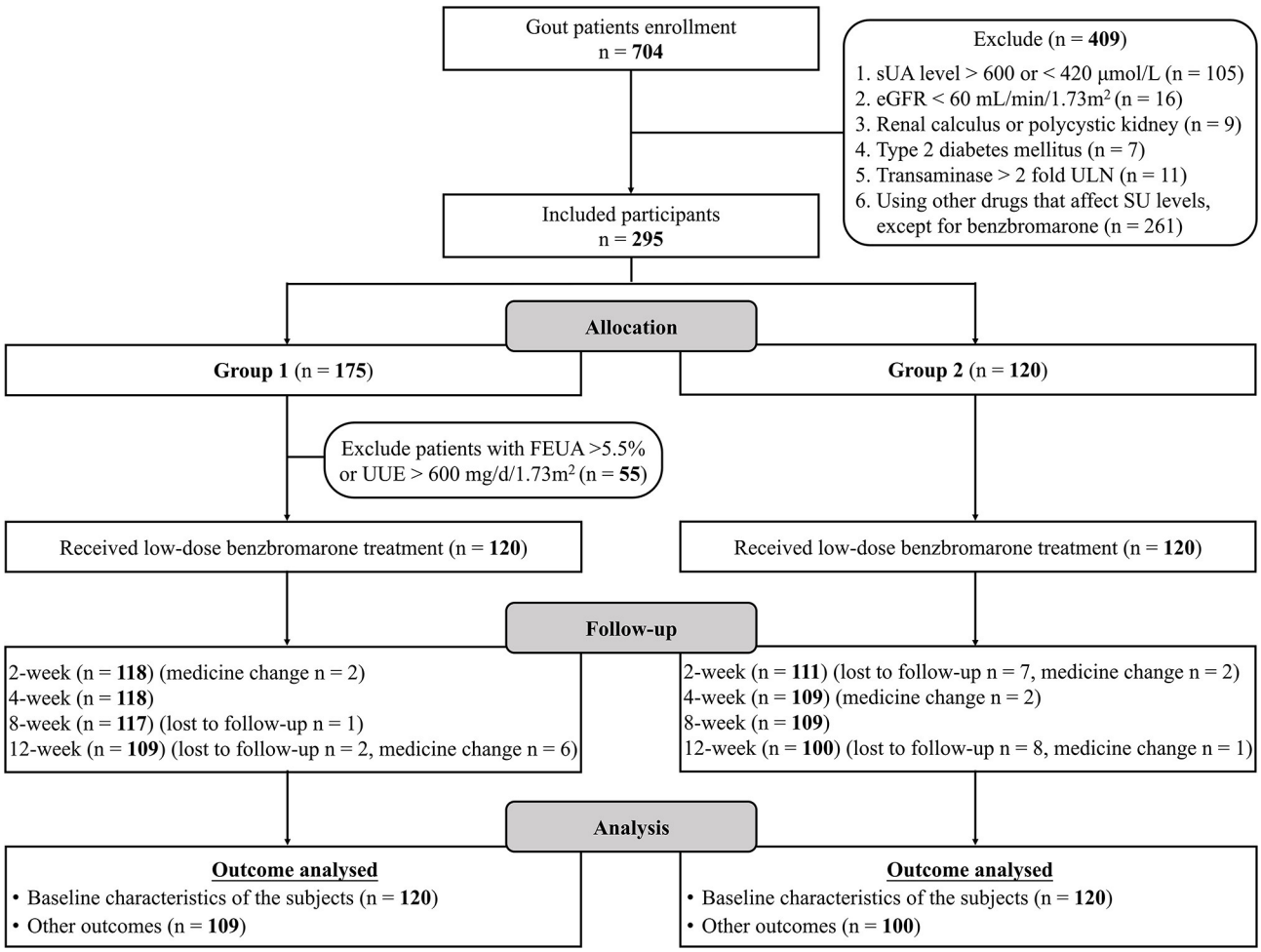

FIGURE 1 | Study flow chart. SU, Serum urate; eGFR, estimated glomerular filtration rate; SU, serum urate; FEUA, fractional excretion of urate; UUE, urinary urate excretion.

if there was evidence of severe adverse effects in the liver or kidney, including increased hepatic enzymes to three times the normal range, eGFR $\leq$ 60 mL/min/1.73 m^2, and any symptoms that indicated poor tolerance to treatment.

Outcomes

The main outcomes were the SU level in each follow-up time point and the proportion of patients achieving the SU target (SU $<$ 360 μmol/L). The frequency of acute gout attack, physical, and chemical indicators (blood and urine routine, liver and kidney function, etc.) were the secondary endpoints. Associations of final SU and clinical variables were analyzed. Safety data were recorded at every follow-up time.

Data Analysis

A total of 117 patients were required for each group to detect a difference in the proportion of patients with SU $<$ 360 μmol/L with a 5% two-sided significance and 90% power based on data from the previous (20). This allows a 10% dropout rate over the period of the study. Since approximately two thirds of patients with gout were found as underexcretors in studies (30), 175 were collected and 120 were allocated to the underexcretion group.

All data were analyzed using the Statistical Product and Service Solutions v22.0 (IBM SPSS, Chicago, USA) software. Clinical characteristics were summarized using standard descriptive statistics including mean (SD), median (interquartile range, IQR), and number (proportion). We employed a mixed model to analyze the variation trend of the two groups with repeated measurement data. The computing methods of this mixed model emphasized the comparison of the changing trend of variables, eGFR, sCr, BUN, TG, and other indicators, between the two groups over the main effect of time. Stepwise linear regression analysis was used to determine the independent predictors associated with SU levels at the final study visit with an inclusion of individual components (urate excretion determination informed, positive gout family history, age, onset age, baseline SU, BMI, and eGFR). Data from regression analyses

were summarized using standardized β coefficients (95% CI). $P < 0.05$ was considered statistically significant.

RESULTS

Patients' Characteristics

There were 704 patients referred for consideration for entry into the study (**Figure 1**). Reasons for exclusion were: SU < 420 μmol/L or SU > 600 μmol/L ($n = 105$), eGFR < 60 mL/min/1.73m^2 ($n = 16$), transaminase > 2-fold of the ULN ($n = 11$), renal calculus or polycystic kidneys ($n = 9$), type 2 diabetes mellitus ($n = 7$), and prescribed drugs affecting SU levels ($n = 261$). We recruited 175 patients for the underexcretion type group and 120 matched patients for the unclassified type group. Clinical typing filtered out 120 underexcretors from 175 patients as 55 with FEUA ≥ 5.5% and/or 24-h UUE > 600 mg/day/1.73m^2. A total of 31 participants were lost to follow-up or adjusted their medication regime. Ultimately, 209 patients (unclassified type group: $n = 100$, underexcretion type group: $n = 109$) completed 12 weeks of ULT and were included in the analysis (**Figure 1**). Baseline characteristics of participants were comparable between the two groups (**Table 1**).

TABLE 1 | Baseline characteristics of the subjects.

	Unclassified type ($n = 120$)	Underexcretion type ($n = 120$)
Demographic and general characteristics		
Age (years), mean (SD)	46.13 (13.51)	44.88 (12.74)
Gout onset age (years), mean (SD)	40.85 (12.22)	39.65 (11.51)
BMI (kg/m^2), mean (SD)	26.16 (2.89)	26.13 (2.87)
Frequency of attacks (per year), median (IQR)	1 (1, 2.5)	1 (1, 2.75)
Coexisting conditions		
Hypertension, n (%)	23 (19.20)	22 (18.30)
Cardiovascular disease, n (%)	8 (6.70)	3 (2.50)
Fatty liver, n (%)	32 (26.70)	21 (17.50)
Hyperlipidemia, n (%)	42 (35.00)	32 (26.70)
Tophus, n (%)	12 (10)	17 (14.20)
Family history of gout, n (%)	30 (26.80)	24 (20.00)
Lifestyles		
Smoking history, n (%)	27 (22.50)	32 (26.70)
Alcohol history, n (%)	47 (39.20)	59 (49.20)
Blood chemistry parameters		
Serum urate (μmol/L), mean (SD)	506.92 (48.60)	499.35 (47.80)
eGFR (mL/min/1.73 m^2), mean (SD)	98.63 (17.03)	98.37 (18.19)
Serum creatinine (μmol/L), mean (SD)	80.43 (10.62)	81.21 (11.45)
Blood urea nitrogen (mmol/L), mean (SD)	4.61 (1.09)	4.18 (0.89)
Triglyceride (mmol/L), mean (SD)	1.84 (0.86)	1.81 (0.84)
Cholesterol (mmol/L), mean (SD)	5.02 (0.97)	4.82 (0.83)
Fasting Glucose (mmol/L), mean (SD)	5.47 (0.54)	5.40 (0.52)
ALT (U/L), median (IQR)	22 (17.25, 33.75)	24 (19, 33)
AST (U/L), median (IQR)	20 (18, 24)	19 (17, 22)

Data were presented as the mean (±SD) or interquartile range.
BMI, body mass index; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range.

Primary Outcomes

Obvious SU reductions were observed after the first 2 weeks of ULT in both the unclassified type group and the underexcretion type group (**Figure 2A**). Further reductions in SU levels were observed at the 4-, 8-, and 12-week time points (**Figure 2A**). The mean (± SD) final SUs were 372.24 (± 55.50) μmol/L in the unclassified type group and 349.73 (± 62.96) μmol/L in the underexcretion group (each $P < 0.001$ compared with baseline) (**Figure 2A**). Compared with the unclassified type group, the underexcretion type group showed significantly lower levels in SU after 4, 8, and 12 weeks ($P < 0.001$, $P < 0.001$, and $P = 0.01$, respectively) (**Figure 2A**).

The proportion of patients who achieved the SU target (SU < 360 μmol/L) increased dramatically after 2 weeks of ULT and remained at this level at the 4-, 8-, and 12-week time points (**Figure 2B**). Significant differences in the percentage of patients who achieved the SU target between the underexcretion type group and the unclassified type group were observed at the 4-week (59.3% *vs.* 45.9%, $P = 0.04$), 8-week (57.1% *vs.* 30.0%, $P < 0.001$), and 12-week time points (60.5% *vs.* 39.0%, $P = 0.002$) (**Figure 2B**).

Secondary Outcomes

No significant changes in the frequency of gout flare, eGFR, sCr, TG, fasting GLU, ALT, or AST were observed during the trial (**Table 2**). However, the levels of BUN increased from 4.65 (± 1.11) mmol/L at baseline to 4.98 (± 1.03) mmol/L at the 12-week time point in the unclassified type group ($P = 0.004$) (**Table 2**). When compared with the unclassified type group, the underexcretion type group had lower levels of TG, TC, and fasting GLU (**Table 2**). Specifically, mean TG in the underexcretion type group was 0.22 mmol/L lower than in the unclassified type group at the 8-week time point ($P = 0.03$) (**Table 2**). Mean TC in the underexcretion type group was 0.31 mmol/L lower than in the unclassified type group at the 8-week time point and 0.30 mmol/L lower at the 12-week time point (both $P = 0.01$) (**Table 2**). Mean fasting GLU levels showed a similar decrease in the underexcretion type group compared with the unclassified type group from the 4th week on and continued until the 12th week ($P = 0.01$, $P < 0.001$, and $P = 0.002$, respectively) (**Table 2**).

Association of Final SU With Clinical Variables in Linear Regression Analysis

During the 12-week follow-up study, no participants were taking drugs (other than benzbromarone) that could influence SU levels. In the entire group, linear regression analysis showed that the factors associated with changes in SU levels at the final study visit were urate excretion determination typing (standardized β: −0.15, 95% CI: −0.29 ∼ −0.02, $P = 0.03$), baseline SU (standardized β: 0.17, 95% CI: 0.03 ∼ 0.31, $P = 0.02$), and BMI (standardized β: 0.20, 95% CI: 0.07 ∼ 0.36, $P = 0.004$) (**Table 3**). For participants in the unclassified type group, only BMI (standardized β: 0.24, 95% CI: 0.03 ∼ 0.50, $P = 0.03$) was an independent predictor of SU, accounting for 5.8% of SU variance

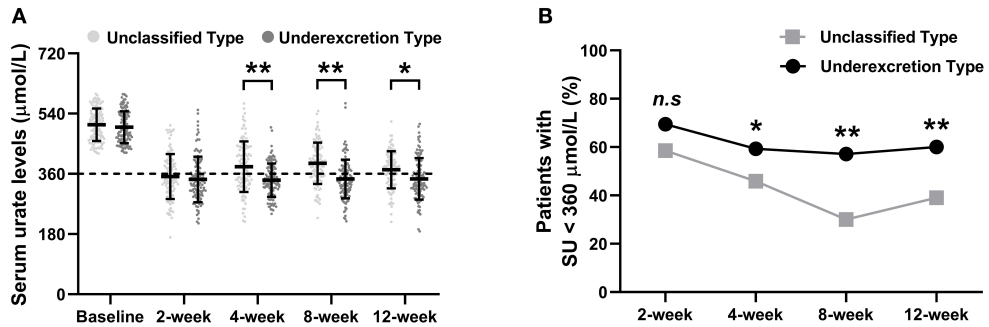

FIGURE 2 | The primary outcomes of the urate-lowering therapy based on hyperuricemia classification. **(A)** The trend of serum urate level and **(B)** proportion of patients with SU < 360 μmol/L during the 12-week study. SU levels are presented as the mean (±SD). Rates are expressed as proportions. SU, serum urate. Symbols * and ** indicate $P < 0.05$ and < 0.01 between the unclassified and underexcretion groups, respectively.

TABLE 2 | Changes in the clinical characteristics during the trial.

	Baseline	4 weeks	8 weeks	12 weeks	*P* trend
Gout flare, n (%)					
Unclassified type	-	11 (9.2)	8 (6.7)	9 (7.5)	0.49
Underexcretion type	-	16 (13.3)	10 (8.3)	13 (10.8)	0.53
P value		0.31	0.62	0.37	
eGFR (mL/min/1.73 m^2), mean (SD)					
Unclassified type	98.63 (17.03)	98.50 (16.11)	97.71 (16.25)	97.26 (15.87)	0.88
Underexcretion type	98.37 (18.19)	98.28 (19.57)	98.02 (18.61)	97.57 (22.98)	0.37
P value	0.81	0.37	0.89	0.91	
Serum creatinine (μmol/L), mean (SD)					
Unclassified type	80.43 (10.62)	80.33 (10.09)	81.02 (10.44)	81.15 (10.18)	0.82
Underexcretion type	81.21 (11.45)	81.03 (13.90)	81.53 (11.47)	82.35 (12.38)	0.79
P value	0.58	0.66	0.72	0.43	
Blood urea nitrogen (mmol/L), mean (SD)					
Unclassified type	4.65 (1.11)	4.64 (1.13)	4.85 (1.22)	4.98 (1.03)	0.004
Underexcretion type	4.18 (0.89)	4.75 (1.26)	4.74 (1.21)	4.89 (1.28)	0.11
P value	0.002	0.50	0.49	0.42	
Triglyceride (mmol/L), mean (SD)					
Unclassified type	1.83 (0.79)	1.90 (0.95)	1.94(1.00)	1.95 (0.89)	0.37
Underexcretion type	1.82 (0.84)	1.78 (0.96)	1.72 (0.92)	1.89(1.41)	0.29
P value	0.63	0.28	0.04	0.83	
Cholesterol (mmol/L), mean (SD)					
Unclassified type	5.02 (0.97)	5.04 (0.91)	5.16 (0.96)	5.14 (0.95)	0.05
Underexcretion type	4.82 (0.83)	4.85 (0.89)	4.85 (0.87)	4.84 (0.85)	0.97
P value	0.09	0.10	0.01	0.01	
Fasting Glucose (mmol/L), mean (SD)					
Unclassified type	5.47 (0.54)	5.55 (0.47)	5.60 (0.47)	5.57 (0.40)	0.28
Underexcretion type	5.40 (0.52)	5.36 (0.66)	5.33 (0.49)	5.39 (0.49)	0.44
P value	0.19	0.01	< 0.001	0.002	
ALT (U/L), median (IQR)					
Unclassified type	22 (17, 34)	23 (17, 33)	23 (17, 33)	22 (17, 30)	0.44
Underexcretion type	24 (19, 33)	23 (18, 33)	24 (17, 33)	23 (17, 31)	0.30
P value	0.50	0.63	0.80	0.76	
AST (U/L), median (IQR)					
Unclassified type	20 (18, 24)	20 (16, 24)	20 (17, 24)	20 (17, 25)	0.40
Underexcretion type	19 (17, 22)	19 (17, 24)	20 (16, 23)	20 (17, 23)	0.13
P value	0.05	0.33	0.64	0.30	

Data were presented as the mean (±SD), interquartile range or numbers (percentage).
BMI, body mass index; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IQR, interquartile range.
P trend value was calculated using a mixed ANOVA model.

TABLE 3 | Predictors of serum urate on the 12-week in linear regression analysis.

	Variable	Standardized β	95% CI	*P*	Model summary
All patients	Urate excretion determination (yes)	−0.15	−0.29∼-0.02	0.03	$R^2 = 0.10$, $F = 7.23$, $P < 0.001$
	BMI	0.20	0.07∼0.36	0.004	
	Baseline SU	0.17	0.03∼0.31	0.02	
Unclassified type	BMI	0.24	0.03∼0.50	0.03	$R^2 = 0.06$, $F = 5.21$, $P = 0.03$
Underexcretion type	BMI	0.22	0.05–0.42	0.02	$R^2 = 0.08$, $F = 4.83$, $P = 0.01$
	eGFR	−0.19	−0.38∼-0.01	0.04	

The following variables were included in the model: baseline SU (60 μmol/L for a unit), urate excretion determination (yes/no), gout family history (yes/no), age, onset age, body mass index, and estimated glomerular filtration rate.
BMI, body mass index; eGFR, estimated glomerular filtration rate.

(**Table 3**), while the independent predictors in the underexcretion type group included eGFR (standardized β: −0.19, 95% CI: −0.38 ∼ −0.01, $P = 0.04$) and BMI (standardized β: 0.22, 95% CI: 0.05 ∼ 0.42, P = 0.02), accounting for 7.8% of SU variance (**Table 3**).

Subgroup Analysis Based on BMI and eGFR

Baseline SU levels, BMI, and urate excretion determination typing were suggested to be predictors, independent of other covariates, of SU at week 12 in the combined cohort, while BMI in the unclassified type group, and baseline BMI and eGFR in the underexcretion type group provided evidence as independent predictors (**Table 3**). We then performed a subgroup analysis based on the BMI (> or ≤ 25 kg/m^2) and eGFR (> or ≤ 90 mL/min/1.73 m^2).

Stratified analysis results revealed a higher proportion of patients with SU < 360 μmol/L in the underexcretion type group than in the unclassified type group, irrespective of the BMI and eGFR grouping, which was manifested by the subgroup of patients with BMI > 25 kg/m^2 and eGFR > 90 mL/min/1.73 m^2 (**Figure 3**). The proportion was significantly higher in the patients with BMI ≤ 25 kg/m^2 than those with BMI > 25 kg/m^2 in the unclassified type group (57.1% *vs.* 25.9%, $P = 0.002$) (**Figure 3A**). In the underexcretion type group, the proportion was higher in the patients with eGFR > 90 mL/min/1.73 m^2 than those with eGFR ≤ 90 mL/min/1.73 m^2 (63.2% *vs.* 43.9%, $P = 0.045$) (**Figure 3B**). The highest proportion of patients in the underexcretion type achieving target SU was observed in patients with eGFR > 90 mL/min/1.73 m^2 and BMI ≤ 25 kg/m^2 (73.1%, **Figure 3C**). A significant gap of 46.1% of the proportion between the underexcretion type and the unclassified type was shown in the subgroup of eGFR > 90 mL/min/1.73 m^2 and BMI > 25 kg/m^2 (**Figure 3C**).

Safety Profile

Safety assessments throughout the study included the incidence and severity of treatment-emergent adverse events (AEs). The proportions of AEs in the two groups were similar while no clinically important or serious AEs were reported (**Supplementary Table 1**).

During the 12-week trial, twenty-five (22.9%) patients had acute gout flares in the unclassified type group and 30 (30.0%) in the underexcretion type group ($P = 0.25$) (**Supplementary Table 1**). In the unclassified type group, 22 (20.2%) patients had one gout attack and 3 (2.8%) had two, while in the underexcretion type group, 22 (22.0%) had one flare, 7 (7.0%) had two, and 1 (1.0%) had more than two (**Supplementary Table 1**).

We measured the serum transaminase levels to assess hepatic safety. No significant changes in transaminase levels were observed over this period (**Supplementary Table 1**). Twenty-one (19.3%) in the unclassified type group and 17 (17.0%) patients in the underexcretion type group had elevated transaminase levels ($P = 0.67$) (**Supplementary Table 1**). During the trial, only five patients' serum transaminase levels were > 2 times upper limit of normal of transaminase, so they were given liver protection and only one withdrew because of > 3 times upper limit of normal of transaminase (**Supplementary Table 1**). After 1 week, the transaminase levels returned to the normal range, which indicated a well tolerance to low-dose benzbromarone.

Assessments of renal safety consisted of eGFR values and renal calculus. Only one patient experienced at least one renal-related AE in the 12-week study, showing eGFR < 60 mL/min/1.73 m^2 (**Supplementary Table 1**). New onset renal calculus was detected using urinary system ultrasound at the end of the trial. Eight (7.3%) patients in the unclassified type group and 6 (6.0%) in the underexcretion type group were reported with renal calculus ($P = 0.70$, **Supplementary Table 1**).

Altogether, three patients were recorded with gastrointestinal disorders, two with increased heart rate compared with before enrollment (80–100/min), one with itchy skin, and one with respiratory, thoracic, and mediastinal disorders in the underexcretion type group (**Supplementary Table 1**).

DISCUSSIONS

To the best of our knowledge, this is the first prospective cohort study to observe the impact of hyperuricemia classification-informed treatment on SU targets in Chinese primary patients with gout. In this clinical trial, we compared the effectiveness of target SU achievement as well as the safety of low-dose

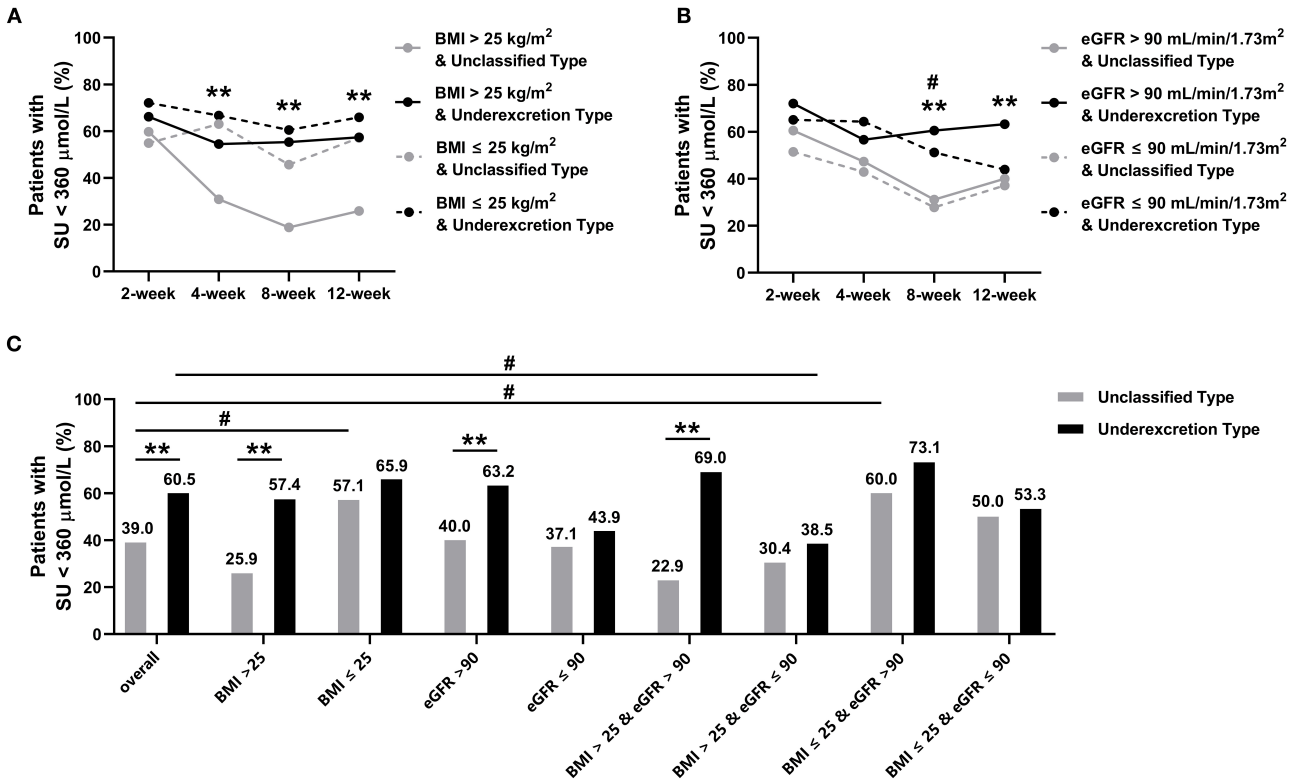

FIGURE 3 | Proportion of patients with serum urate (SU) < 360 μmol/L in subgroups during the 12-week study. **(A)** Subgrouped by BMI (>25 kg/m^2 or ≤ 25 kg/m^2), symbols * and ** indicate P <0.05 and <0.01 in BMI > 25 kg/m^2 between unclassified and underexcretion groups, respectively. **(B)** Subgrouped by eGFR (> 90 mL/min/1.73 m^2 or ≤ 90 mL/min/1.73 m^2), symbols * and ** indicate P <0.05 and <0.01 in eGFR > 90 ml/min/1.73 m^2 between unclassified and underexcretion groups, respectively; symbols # and ## indicate P <0.05 and <0.01 between eGFR ≤ 90 ml/min/1.73 m^2 in unclassified and underexcretion groups, respectively. **(C)** Subgrouped by BMI and eGFR (BMI > 25 kg/m^2 and eGFR > 90 mL/min/1.73 m^2, BMI > 25 kg/m^2 and eGFR ≤ 90 mL/min/1.73 m^2, BMI ≤ 25 kg/m^2 and eGFR > 90 mL/min/1.73 m^2, BMI ≤ 25 kg/m^2 and eGFR ≤ 90 mL/min/1.73 m^2), symbols * and ** indicate P < 0.05 and < 0.01 between unclassified and underexcretion groups within same subgroups, respectively, symbol # indicates P < 0.05 between overall and subgroups within unclassified or underexcretion groups. eGFR, estimated glomerular filtration rate; BMI, body mass index; SU, serum urate.

benzbromarone between a group of patients of the unclassified excretion type and those classified as underexcretion type for 12 weeks. Notably, the target SU was achieved by 60.5% of underexcretors compared with 39.0% of patients of the unclassified excretion type group at the 12th week ($P = 0.002$) without significant clinically important or serious AEs. Our data support the classification of HU in the treatment of gout, with improved proportions of achieving target SU.

Currently, the rate of successfully achieving the target SU level remains suboptimum, showing 37.5% in a Japanese cross-sectional study (31) and 35.7% in our previous clinical trial in which patients were treated with low-dose benzbromarone (20). Besides, a meta-analysis involving 137,699 patients with gout revealed that the overall adherence rate was only 47% (95% CI: 42%∼52%, $I^2 = 99.7\%$) (32). These data may reflect poor compliance or non etiological target treatment. The worth of classification of hyperuricemia/gout has not been widely recognized although the 2006 EULAR gout guidelines and 2018 management consensus on hyperuricemia/gout proposed by Taiwan experts acknowledged the necessity of renal urate excretion determination (22, 23). Previous studies have indicated that for underexcretors, patients taking benzbromarone 100 mg/day showed a 58.27% decrease of percentage from initial SU compared with only 36.26% in patients taking allopurinol 300 mg/day (17). Another study also confirmed that low dose (40 mg) febuxostat was more effective in patients with urate overproduction than in underexcretors (19).

So far, renal urate underexcretion has been widely considered to be a main cause of hyperuricemia (30). Although the unclassified type group might contain a majority of underexcretors, the differences in SU decrease and proportion of SU < 360 μmol/L were significant between the groups in our study. The increased proportions of patients achieving SU target in the underexcretion type group at each follow-up time point indicated a high effectiveness of low-dose benzbromarone for these patients. Our data showed a 1.5-fold increase in the proportion of patients achieving target SU, further confirming the advantages of hyperuricemia classification typed drug selection and showing an evidence-based suggestion in clinic.

In addition, our study also found that patients in the underexcretion type group had significantly lower blood glucose and cholesterol after 8-week ULT and afterward compared with

those in the unclassified type group. This result can be explained by the fact that fasting glucose was positively correlated to SU when it is below the threshold (6.5 mmol/L in men and 7.5 mmol/L in women) (33). In addition, the indirect mechanism of insulin resistance may also be involved in the results (34, 35).

Association by linear regression analysis of all enrollments showed that patients in the underexcretion type group were more likely to reach the target of SU than those in the unclassified type group. Our study found that baseline SU level was a predictor independent of included covariates for the final SU level in all participants. This is consistent with our previous research (20). In addition, the final SU level in the unclassified type group was also associated with BMI at baseline, while in the underexcretion type group was also associated with eGFR at baseline. It has been reported that BMI is a risk factor for hyperuricemia independent of covariates (36). Moreover, Yamashita et al. (37) showed an association between BMI and renal handling of urate, suggesting that hyperuricemia in obese people is mainly attributed to an impaired renal clearance of urate rather than overproduction. An association between a high BMI and a lower FEUA has been evidenced in Eastern Polynesian and New Zealand European participants as well (15). The stratified analysis of eGFR showed that the proportion of achieving SU target in patients with eGFR $>$ 90 mL/min/1.73 m^2 was significantly higher than that of patients with eGFR $\leq$ 90 mL/min/1.73 m^2 in the underexcretion type group. This can be explained by the fact that a strong renal function indicates a good renal handling of urate (38).

The latest ACR gout diagnosis and treatment guidelines further emphasize the importance of reaching the target of SU and the necessity of taking lifelong urate-lowering medication. Therefore, from the consideration of long-term maintenance of target SU, there is an ongoing need to lower the level of SU more effectively. We strongly recommend that the classification for type-leading treatment is etiologically consistent and abides by the principle of precision therapy. The urate-lowering efficacy was comparable between 25 mg benzbromarone qd. and 20 mg febuxostat qd. (20). Fujimori et al. (39) suggest that benzbromarone is applicable to the management of hyperuricemia associated with renal impairment even with long-term use.

We acknowledge the limitations of this study. Our study was a single-center clinical trial and should be confirmed in multiple centers. The results may not be generalizable to other ethnic groups and to people living in other countries. Currently, we only compared the efficacy of benzbromarone administration, in the absence of a comparison with allopurinol or febuxostat.

CONCLUSIONS

In summary, this study has demonstrated the positive effect of clinical classification of gout on SU targets. High effectiveness without significant treatment-emergent AEs was concluded in the urate underexcretion group when using low-dose benzbromarone as the urate-lowering regime. These data provide further support for classification application in the type-leading treatment of patients with gout and provide evidence for the amendments of guidelines. Therefore, we propose that patients with gout need to be tested for clinical classification when choosing urate-lowering drugs. It was recommended that low-dose benzbromarone should be the first-line drug in urate underexcretors without renal insufficiency or other contraindications.

AUTHOR'S NOTE

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

CL designed the study with XX and JL. CL and JL had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. XX, JL, and TM were major contributors in writing the manuscript. XX, XY, XL, LC, ZL, WS, LH, FY, YH, AJ, and CW have worked on the sample processing. XY, JL, XX, and TM contributed in the interpretation of data. XX, XY, and LH contributed equally to this article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.806710/full#supplementary-material

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REVIEW
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Current State and Issues of Regenerative Medicine for Rheumatic Diseases

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The prognosis of rheumatic diseases is generally better than that of malignant diseases. However, some cases with poor prognoses resist conventional therapies and cause irreversible functional and organ damage. In recent years, there has been much research on regenerative medicine, which uses stem cells to restore the function of missing or dysfunctional tissues and organs. The development of regenerative medicine is also being attempted in rheumatic diseases. In diseases such as systemic sclerosis (SSc), systemic lupus erythematosus (SLE), and rheumatoid arthritis, hematopoietic stem cell transplantation has been attempted to correct and reconstruct abnormalities in the immune system. Mesenchymal stem cells (MSCs) have also been tried for the treatment of refractory skin ulcers in SSc using the ability of MSCs to differentiate into vascular endothelial cells and for the treatment of systemic lupus erythematosus SLE using the immunosuppressive effect of MSCs. CD34-positive endothelial progenitor cells (EPCs), which are found in the mononuclear cell fraction of bone marrow and peripheral blood, can differentiate into vascular endothelial cells at the site of ischemia. Therefore, EPCs have been used in research on vascular regeneration therapy for patients with severe lower limb ischemia caused by rheumatic diseases such as SSc. Since the first report of induced pluripotent stem cells (iPSCs) in 2007, research on regenerative medicine using iPSCs has been actively conducted, and their application to rheumatic diseases is expected. However, there are many safety issues and bioethical issues involved in regenerative medicine research, and it is essential to resolve these issues for practical application and spread of regenerative medicine in the future. The environment surrounding regenerative medicine research is changing drastically, and the required expertise is becoming higher. This paper outlines the current status and challenges of regenerative medicine in rheumatic diseases.

Keywords: stem cell transplantation (SCT), rheumatic diseases (RDs), therapeutic angiogenesis, regenerative medicine, hematopoietic stem cell (HSC), mesenchymal stem cell (MSC), endothelial progenitor cell (EPC)

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INTRODUCTION

Stem cells play an important role in maintaining the structure and function of tissues in our bodies by replenishing old cells with new ones (1). Stem cells have both the ability to divide into cells with the same ability as themselves (self-renewal capacity) and the ability to differentiate into various types of cells (multipotentiality). In recent years, stem cell transplantation (SCT) has been applied to various fields of regenerative medicine by utilizing the properties of stem cells (**Table 1**).

TABLE 1 | Comparison of the properties of cells used in regenerative medicine.

	ESC	iPSC	HSC/MSC	EPC
Origin	Embryo	Transgenic somatic cells	Adult tissues	Adult tissues
Differential potential	Pluripotent	Pluripotent	Multipotent	Unipotent
Differentiated cells	All cells	All cells	Limited types of cells	Vascular endothelial cells
Proliferation potential	Very high	Very high	Not high	Low
Allogenic/Autologous	Allogenic	Both	Both	Autologous
Rejection	Possible	• Allogenic: Possible • Autologous: Unlikely	• Allogenic: Possible • Autologous: Impossible	Impossible
Ethical issues	Many	Few	None	None
Clinical issues	Risk of tumorigenesis, Instability of supply	Risk of tumorigenesis, Difficulty in quality control	Nothing particular	Nothing particular
Clinical application status	None	Under clinical trial	Widespread	Under clinical trial

ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; EPC, endothelial progenitor cell.

The prognosis of rheumatic diseases is generally better than that of malignant tumors, and they have a chronic course. However, there are some patients with poor prognoses who show resistance to conventional therapy and develop irreversible functional impairment and organ damage. Since the mid-1990's, mainly in Europe and the United States, hematopoietic stem cell transplantation (HSCT) has been tried for such patients with poor prognosis, with the aim of correcting abnormalities of the immune system and reconstructing it (2). HSCT is expected to be widely used in daily clinical practice also in the field of rheumatology.

Mesenchymal stem cells (MSCs) are also attracting attention in regenerative medicine, such as the treatment of intractable skin ulcers in systemic sclerosis (SSc) using the differentiation ability of MSCs into vascular endothelial cells and the treatment of systemic lupus erythematosus (SLE) using the immunosuppressive effect of MSCs (3, 4). Endothelial progenitor cells (EPCs) contained in the mononuclear cell fraction of bone marrow and peripheral blood also can differentiate into vascular endothelial cells (5), and their application to vascular regeneration therapy is being attempted. In addition, induced pluripotent stem cells (iPS cells), which have been recently discovered, are expected to be applied to rheumatic diseases (6). In this paper, we review the current status and challenges of regenerative medicine using stem cells in rheumatic diseases.

HEMATOPOIETIC STEM CELL TRANSPLANTATION

Among the various types of stem cells, somatic stem cells, which are present in our body, are the closest to practical use in regenerative medicine. In 1957, Thomas et al. performed the world's first transplantation of hematopoietic stem cells (HSCs) from the bone marrow of a healthy donor to a patient with leukemia after anticancer drugs and total-body irradiation (7). Since then, bone marrow transplantation (BMT) has been attempted in many countries around the world, and technological advances through trial and error have greatly improved treatment outcomes. Furthermore, it has been shown that HSCs are present not only in bone marrow but also in peripheral blood and cord blood. Since the late 1980's, allogenic or autologous peripheral blood stem cell transplantation (PBSCT) and allogeneic cord blood transplantation (CBT) have also been applied clinically (8–10). Thus, hematopoietic stem cell transplantation (HSCT) is now a well-established and widespread therapy.

The efficacy of HSCT in correcting and reconstructing immune abnormalities in autoimmune diseases has been previously demonstrated in various animal models of disease (11). In spontaneous autoimmune disease models such as MRL/*lpr* mice, only allogeneic transplantation can suppress the onset of disease and induce remission (12–15), whereas, in antigen-induced autoimmune disease models, both allogeneic and syngeneic transplantation can induce remission (16–18). It is thought that human autoimmune diseases are not caused by genetic predisposition alone but also by environmental factors as well. The result that remission induction was possible even with syngeneic transplantation in the antigen-induced models suggests the possibility of clinical application of autologous PBSCT in autoimmune diseases. In addition, case reports of improvement of autoimmune diseases associated with hematological disorders by HSCT have been accumulated (19).

With this background, the application of HSCT to autoimmune diseases such as SSc, SLE, and rheumatoid arthritis (RA), which are refractory to conventional therapy, has been attempted since the mid-1990's, mainly in Europe and the United States. A total of 3,320 transplant procedures, including 1,634 patients with multiple sclerosis, 652 patients with SSc, 196 patients with Crohn's disease, 168 patients with inflammatory arthritis, and 110 patients with SLE, were registered in the autoimmune disease database of the Blood and Marrow Transplantation (EBMT) from 1994 to 2019 (20). Three thousand one hundred three patients received a first autologous HSCT, and 217 patients received an allogeneic HSCT. The number of procedures per year has increased in recent years, especially in multiple sclerosis and SSc, while other indications such as inflammatory arthritis and SLE have decreased considerably. Analysis of the follow-up period showed a 5-year overall survival

rate of 86%, a progression-free survival rate of 49%, a relapse incidence rate of 46%, and non-relapse mortality of 5.3% (21).

Many phase I/II trials have been conducted for SSc (**Table 2**). The results of HSCT (PBSCT 55 patients, BMT 2 patients) performed on 57 SSc patients enrolled in the registry of EBMT and European League against Rheumatism (EULAR) showed significant improvement in skin sclerosis (22). There are three randomized controlled trials for HSCT in SSc, and most of the data show significant improvement in patient's skin scores and moderate improvement in FVC and DLCO. In an open-label randomized phase II trial (Autologous Non-myeloablative Hematopoietic Stem-cell Transplantation Compared with Pulse Cyclophosphamide Once per Month for SSc; ASSIST), 19 SSc patients with organ involvement received HSCT or 6-month monthly IVCY, Eighty-nine percent of the patients who received IVCY progressed within 1 year of randomization, while none of the patients who received HSCT progressed (23). Seven with confirmed progression on IVCY received an HSCT. Eleven patients who received HSCT and were followed for at least 2 years showed significant improvement in skin score and FVC as compared with baseline. In a European multicenter randomized open-label phase III study comparing autologous PBSCT with intravenous cyclophosphamide (IVCY) in 156 patients with SSc (Autologous Stem Cell Transplantation International Scleroderma; ASTIS), treatment-related mortality at 1 year was higher in the PBSCT group than in the IVCY group (10 vs. 0%), but mortality at 4 years was lower in the PBSCT group (hazard ratio 0.29) (24). In a similar randomized open-label phase II trial of 75 SSc patients in the United States (Scleroderma Cyclophosphamide Or Transplantation; SCOT), treatment-related mortality at 6 years was higher in the PBSCT group than in the IVCY group (6 vs. 0%) while the PBSCT group was superior to the IVCY group in terms of asymptomatic survival (74 vs. 47%) and overall survival (86 vs. 51%), respectively (25). Improvements in screening methods and transplantation techniques have reduced transplanted-related mortality from 10% in the ASTIS trial (24) to 3% in the SCOT trial (25, 26). In a recent EBMT multicenter prospective non-interventional study, higher baseline skin score and older age at transplantation were associated with lower progression-free survival, and CD34+ selection was associated with better response (27). By the accumulation of various evidence, autologous HSCT is now an essential part of the SSc treatment and is supported by the latest recommendations of the EULAR; the recommendations state that HSCT should be considered as a treatment for skin and lung disease in selected patients with rapidly progressive SSc at risk of organ failure (strength of recommendation: (A) (28). Regarding the mechanism by which PBSCT exerts its effects, there are reports that (1) it corrects the Th2 bias of the Th1/Th2 balance in SSc (29), (2) it increases naïve T cells and decreases central memory T cells in peripheral blood *via* thymic reactivation (30), and (3) it maintains self-tolerance by increasing regulatory T cells in peripheral blood (31).

In SLE, HSCT has been performed for refractory or life-threatening cases of SLE, and several phase I/II trials demonstrated disease remission rates of around 30 to 70% at 5 years and significant improvements in quality of life (**Table 2**) (32–35). In a single-center prospective study of autologous PBSCT in seven patients with SLE refractory to standard therapy, clinical remission was achieved once in all seven patients, and five patients showed no clinical or serological evidence of SLE activity during a median follow-up of 60 months (32). A single-arm trial of 50 SLE patients refractory to standard therapies and either organ- or life-threatening involvement was conducted, and autologous non-myeloablative PBSCT showed promising results for the SLE Disease Activity Index (SLEDAI) score and serum markers, stabilized nephropathy, and prolonged disease-free survival at 5 years (50%) (33). EBMT registry also showed that autologous PBSCT improved SLEDAI in 28 patients, with an overall survival rate of 81% and disease-free survival rate of 29% at 5 years (34). Autologous PBSCT in patients with SLE refractory to conventional therapy resulted in disease remission of 92% at 1 year and 62% at 5 years after transplantation in 26 patients treated with cyclophosphamide, rabbit ATG, and rituximab as non-myeloablative therapy (35).

In RA, some successful studies of HSCT in RA were reported, but the results were not encouraging considering the cost-benefit balance (**Table 2**) (36–39). Although progression-free survival and disease-free survival were high in patients with RA compared to other rheumatic diseases, the high relapse rate and the development of biologic agents did not justify the further development of HSCT in RA.

The main advantage of HSCT for rheumatic diseases is the ability to reset the immune system and alter the natural history of the disease by removing T-cell clones involved in the autoimmune response. On the other hand, the main disadvantage of HSCT is the added toxicity to the body from the high doses of chemotherapy and radiation used as part of the conditioning regimen. Future challenges for HSCT in rheumatic diseases include how to reduce transplant-related mortality in terms of the risk-benefit balance of treatment. To this end, improvements should be made in the following areas: optimization of indication criteria, optimization of HSC collection, the necessity of CD34 cell purification, and optimization of pre-transplant procedures.

As for indications for HSCT, the EBMT published its recommendations on indications for HSCT in 2015 (40). This was developed based on prospective clinical trials, registry data, and expert opinion. In patients with SSc, HSCT is indicated for a specific subgroup of patients (those with non-Reynaud's disease for < 5 years, modified Rodnan skin score > 15, and major respiratory, cardiac, or renal involvement with documented evidence of onset or clinically significant deterioration within the past 6 months). In patients with SLE, HSCT is optional for certain subgroups of patients (early in the disease course or after at least 6 months of standard therapy with mycophenolate mofetil or cyclophosphamide with persistent or relapsed disease activity as defined by the British Isles Lupus Assessment Group (BILAG) A score with cardiovascular, neurologic, renal, or pulmonary involvement, vasculitis, or autoimmune cytopenia. The American Society for Blood and Marrow Transplantation (ASBMT) has established a multi-stakeholder task force composed of transplant professionals, insurance

TABLE 2 | Clinical trials using regenerative medicine for rheumatic diseases.

Intervention	Comparison	Disease	Study phase	Patient number	Treatment effect	Treatment toxicity	References
Autologous PBSCT/BMT	-	SSc	I/II	57	Improvement in skin sclerosis at 6–36 months	Treatment-related mortality 8.7%	(22)
Autologous PBSCT	IVCY	SSc	II	19 (10 vs. 9)	Improvement in skin sclerosis and pulmonary function at 12 months (vs. IVCY)	No deaths	(23)
Autologous PBSCT	IVCY	SSc	III	156 (79 vs. 77)	Improvement in skin sclerosis and pulmonary function at 2 years (vs. IVCY), Improvement in event-free survival at 1–10 years (vs. IVCY)	Treatment-related mortality 10.1% at 1 year	(24)
Autologous PBSCT	IVCY	SSc	II	75 (36 vs. 39)	Improvement in the global rank composite score and event-free survival at 54 months (vs. IVCY)	Treatment-related mortality 3% at 54 months and 6% at 72 months	(25)
Autologous PBSCT	-	SLE	I/II	7	Achievement of clinical remission in all cases, Disappearance of serum anti-dsDNA antibody in all cases within 1 month	One death due to invasive central nervous system aspergillosis at 3 months	(32)
Autologous PBSCT	-	SLE	II	50	Decrease in disease activity at 6 months to 5 years, Improvement in pulmonary function at 12–60 months	Treatment-related mortality 2%	(33)
Autologous PBSCT	-	SLE	II	32	Decrease in disease activity at 6 months to 5 years	No treatment-related deaths	(35)
Autologous PBSCT (Unmanipulated cells)	Autologous PBSCT (CD34-selected cells)	RA	II	33 (15 vs. 18)	Decrease in disease activity over a median follow-up of 167 (range 45–374) days (Similar outcomes in both groups)	No deaths	(37)
Autologous PBSCT	-	RA	I/II	14	Decrease in disease activity at 3–12 months	No deaths	(38)
Allogeneic UC-MSCT	-	SLE	I/IIa	16	Decrease in disease activity at 1–24 months	No deaths	(70)
Allogeneic BM-/UC-MSCT	-	SLE	I/II	81	Improvement in renal function and decrease in disease activity at 12 months	No deaths	(71)
Autologous SVF transplantation	-	SSc	I	12	Improvement in hand disability, pain, Raynaud's phenomenon, finger edema and quality of life at 6 months	No deaths	(76)

PBSCT, peripheral blood stem cell transplantation; BMT, bone marrow transplantation; SSc, systemic sclerosis; IVCY, intravenous cyclophosphamide; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; UC-MSCT, umbilical cord-derived mesenchymal stem cell transplantation; BM-MSCT, bone marrow-derived mesenchymal stem cell transplantation; SVF, stromal-vascular fraction.

company representatives, and patient advocates to guide "routine" indications for HCT (41). Recommendation categories of allogeneic HSCT for SSc, SLE, and RA are "Not generally recommended," while those of autologous HSCT are "Developmental." The guidelines published by the Japanese Society for Hematopoietic Cell Transplantation also describe the current indication criteria (42). The guideline limits eligible patients to those with life-threatening conditions due to intractable disease, or those whose quality of life is significantly reduced due to sequelae even if the disease itself can be controlled. It also describes the criteria for HSCT indications in each rheumatic disease. The number of institutions with experience in HSCT for rheumatic diseases in Japan is still small, and it is important to accumulate experience in transplantation in the future. It is important to accumulate transplantation experience in the future. For this purpose, it is necessary to strengthen cooperation between rheumatologists and hematologists.

MESENCHYMAL STEM CELL TRANSPLANTATION

Mesenchymal stem cells (MSCs) are somatic stem cells with the ability to differentiate into a variety of tissues and have recently attracted attention as a cell source for regenerative medicine (43, 44) (**Table 1**). In 1970, Friedenstein et al. (45) revealed the existence of MSCs in guinea pigs as cells with the ability to differentiate into bone. Isolation and culture expansion of human bone marrow MSCs was reported in 1992 (46), and the safety of bone marrow MSC injection into patients was reported as early as 1995 (47). In 1999, Pittenger et al. (48) reported MSCs

from human bone marrow as cells with the ability to differentiate into bone, fat, and cartilage. MSCs have been found in various connective tissues such as adipose tissue (49, 50), umbilical cord (51), placenta (52, 53), synovium (54, 55), and dental tissues (56, 57) in addition to bone marrow (46, 48), and are thought to play an important role in repair and homeostasis in each tissue. MSCs can differentiate not only into mesenchymal cells such as bone, cartilage, and adipocytes but also into tissue cells of ectodermal origin such as neurons and endodermal origin such as hepatocytes (58–61).

Clinical trials of regenerative medicine using MSCs have been actively conducted around the world, and the U.S. National Library of Medicine's clinical research database "ClinicalTrials.gov" has registered about 1,300 clinical trials as of November 2021. In 2011, Hearticellgram®-AMI, autologous bone marrow-derived MSCs (BM-MSCs), was approved in Korea as the world's first stem cell therapy for acute myocardial infarction (62). Subsequently, several stem cell drugs, such as MSC-rich cryopreserved placental membrane for the treatment of diabetic foot ulcers (63) and allogeneic BM-MSCs for the treatment of acute GVHD utilizing their immunosuppressive function (64, 65), have also been developed and marketed. Recently, Japan has conditionally approved an autologous BM-MSC drug for the treatment of spinal-cord injury although some researchers believe that this approval is premature (66).

In 2008, the first case of BM-MSC transplantation for the treatment of rheumatic diseases was reported by a German group (67). The patient had severe progressive diffuse cutaneous SSc seropositive for anti-Scl-70 antibodies, and digital ulcers and skin sclerosis improved after intravenous injection of allogeneic BM-MSCs. Later, the same group reported that skin sclerosis and limb ulcers improved in 4 of 5 SSc patients who underwent allogeneic BM-MSC transplantation, and no serious adverse events were observed (68). In addition, there is a report that autologous BM-MSC transplantation in SSc patients with gangrene improved blood flow in the extremities on angiography, and areas of necrotic skin were reduced (69).

MSC transplantation has been attempted in SLE patients because of the immunosuppressive effect of MSCs (**Table 2**). A Chinese group reported that umbilical cord-derived MSC (UC-MSC) transplantation in 16 patients with SLE refractory to standard therapy resulted in significant improvement in SLEDAI and renal function at a median observation period of 8 months (70). Another report by the same group showed that 81 patients with Class III, IV, or V lupus nephritis refractory to immunosuppressive therapy received intravenous allogeneic BM- or UC-MSCs, and about 60% of the patients achieved renal remission after 12 months (71).

In the past, bone marrow was mainly used as a source of MSCs. However, adipose-derived MSCs (ASCs) have been widely used in regenerative medicine because adipose tissue contains a large amount of MSCs and they can be easily obtained from subcutaneous adipose tissue by liposuction (72). Because ASCs can differentiate into vascular endothelial cells and improve blood circulation in a mouse model of hindlimb ischemia (73), ASC transplantation for critical limb ischemia has been tried and the data demonstrated the feasibility and safety (74). In SSc, an Italian group reported that subcutaneous transplantation of autologous ASCs and hyaluronic acid in 6 cases resulted in improvement of skin sclerosis in all cases at 1 year with no adverse events (75). In addition, a French group subcutaneously injected stromal-vascular fraction (SVF) containing autologous ASCs in 12 patients with SSc and found that skin thickness, finger edema, Raynaud's phenomenon, hand disability, pain, and quality of life improved at 6 months after administration without any severe adverse events (76).

MSCs are expected to be effective in regenerative medicine in a wide range of fields including rheumatic diseases, and various clinical studies have been conducted. However, most of these clinical studies have shown short-term improvement in clinical symptoms, and the number of cases is still small. In the future, it is necessary to investigate the long-term clinical effects of MSCs in larger-scale clinical studies to establish the evidence. In addition, the standardization and optimization of technologies for regenerative medicine using MSCs will also be an issue in the future.

REGENERATIVE MEDICINE USING INDUCED PLURIPOTENT STEM CELLS

Pluripotent stem cells are stem cells that can differentiate into any type of cells, unlike somatic stem cells, and include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). In 1981, two independent groups, Evans et al. and Martin et al., established ESCs in mice (77, 78). In 1998, Thomson et al. (79) established the world's first human ESCs. The problem of immune rejection has been a barrier to the application of ESCs in regenerative medicine (**Table 1**). More importantly, bioethical issues have always been a concern in research and medical treatment using ESCs as these cells are generated from fertilized eggs (80).

In 2006, Takahashi et al. (6, 81) established the world's first iPSCs from mouse fibroblasts, and iPSCs were also established from human fibroblasts in 2007. iPSCs are generated from mature somatic cells and do not pose the problems of both rejection response and bioethics as pointed out for ESCs (**Table 1**), so research on regenerative medicine using iPSCs has rapidly expanded in recent years. In 2014, a research group led by RIKEN and others conducted the world's first iPSC-based regenerative therapy for exudative age-related macular degeneration, in which retinal pigment epithelium derived from the patient's own iPSCs was transplanted under the retina, and confirmed its long-term efficacy (82, 83). Subsequently, a case was reported in which a patient with idiopathic Parkinson's disease was transplanted with midbrain dopaminergic progenitor cells differentiated *in vitro* from autologous iPSCs, and Parkinson's symptoms improved (84). Now clinical trials of regenerative medicine using autologous iPSCs for various disorders, such as thrombocytopenia, recessive epidermolysis bullosa dystrophica, and muscular dystrophy are also undergoing or planned.

iPSCs are expected to be the starting point for personalized medicine with autologous cell therapy. In contrast to allogeneic cell therapies, autologous iPSCs therapies can treat a variety of

diseases without the need for immunosuppression. Autologous iPSCs therapies can be used to treat all patients, not just those not covered by HLA haplobanks. At present, there is no information on the development of regenerative medicine using iPSCs in rheumatic diseases. Ikuno et al. (85) at Kyoto University have developed a technology to efficiently generate vascular endothelial cells from human pluripotent stem cells, which is expected to be applied to vascular regeneration therapy for limb ischemia associated with rheumatic diseases such as SSc. Basic research on the regeneration of cartilage using iPSCs is in progress and may be applied to patients with rheumatoid arthritis whose cartilage is damaged (86, 87).

In human trials involving transplantation of autologous iPSC-derived cells, the longest follow-up has been reported to be 4 years after transplantation, and so far, no serious side effects have been reported, although the number is limited (82–84). Nonetheless, there are still many issues to be solved in regenerative medicine using iPSCs. The formation of teratomas due to the contamination of undifferentiated iPSCs, the possibility of cancerization due to genetic damage during the process of iPSC generation and cultivation, and the development of technology to obtain a sufficient amount and quality of cells for transplantation and a tissue structure suitable for transplantation are major issues for the future. As it takes several months to reprogram and differentiate somatic cells harvested from a patient to produce a cell therapy for the patient (88), optimizing this manufacturing timeline would help ensure that patients receive iPSC-based therapies in a timely manner.

THERAPEUTIC ANGIOGENESIS USING VASCULAR ENDOTHELIAL PROGENITOR CELLS

In 1997, it was discovered that a fraction of adult peripheral blood mononuclear cells (PBMCs) differentiate into vascular endothelial cells in culture and was named endothelial progenitor cells (EPCs) (5). EPCs are positive for the surface antigen CD34 and are a distinct population from MSCs, which are negative for CD34. From the standpoint of embryology and histology, neovascularization can be divided into two types: (1) vasculogenesis, in which hematopoietic stem cells (HSCs) and vascular endothelial cells differentiate from blood islands composed of hemangioblasts in early embryonic stages to form primitive blood vessels, and (2) angiogenesis, in which existing vascular endothelial cells undergo sprouting and migration (89). In the past, it was thought that angiogenesis in adults depended solely on angiogenesis. However, it was revealed that EPCs are present in the circulating blood of adults and are involved in the development of new blood vessels, suggesting that vasculogenesis, which was thought to exist only in the fetal period, may also be established in the adult (5). Subsequent studies revealed that EPCs originate from the bone marrow and are mobilized into the peripheral blood and incorporated into angiogenic sites as needed (90, 91).

From these cellular characteristics, basic research on vascular regeneration therapy was conducted using an animal model of lower limb ischemia. Intravenous administration of cultured human peripheral blood EPCs to immunodeficient mice model of lower limb ischemia improved blood flow in the limb due to angiogenesis (92). In addition, transplantation of autologous bone marrow mononuclear cell (BM-MNC) fractions, which contain EPCs, into the ischemic limb of a rabbit model of lower limb ischemia resulted in angiogenesis and improved blood flow in the limb (93), demonstrating the efficacy of vascular regeneration therapy using BM-MNCs or EPCs.

Based on the results of the basic research described above, a multi-center clinical study of Therapeutic Angiogenesis by Cell Transplantation (TACT) was conducted, in which bone marrow was harvested from the iliac bone of patients with critical limb ischemia and BM-MNC fractions containing EPCs were isolated and transplanted into the ischemic site. When patients with bilateral lower limb ischemia were randomly injected with BM-MNCs in one lower limb and PBMCs in the other limb as a control, the ankle-brachial index, transcutaneous oxygen pressure, rest pain, and pain-free walking time of the limb infused with BM-MNCs improved significantly at 4 weeks as compared to the limb infused with PBMCs (94). In a long-term efficacy and safety study, patients with arteriosclerosis obliterans or thromboangiitis obliterans were implanted with BM-MNCs and maintained significant improvements in leg pain scale, ulcer size, and pain-free walking distance for at least 2 years after treatment (95–97). In the group of patients who responded to the treatment, the number of circulating $CD34^+$ and $CD133^+$ cells increased continuously for 1 month after treatment, while the number did not increase in the non-responder group (98).

It has been reported that the absolute number of circulating EPCs and their ability to differentiate into vascular endothelial cells are reduced in patients with SSc (99). Thus the therapeutic angiogenesis using autologous BM-MNCs was applied to eight SSc patients with refractory skin ulcers and found that all ulcers disappeared within 6 months, and the improvement of pain was also excellent (100). As the results of therapeutic angiogenesis using autologous BM-MNCs in 69 patients (39 SSc, 30 other collagen diseases) with severe ischemic limb caused by collagen disease accumulated in the TACT study, the 10-year overall survival rate, major amputation-free rate, and amputation-free survival rate were 67.6, 90.9, and 61.2%, respectively with the adverse events occurred in 8.7% (101). The 10-year major amputation-free rates were 97.4% in the SSc group and 82.6% in the other collagen disease group, and the rate of limb salvage tended particularly to be higher in the SSc group among the collagen diseases (102).

The efficacy of transplantation of mononuclear cell fractions containing EPCs in peripheral blood mobilized from bone marrow by subcutaneous injection of G-CSF into the ischemic region is also currently being investigated. Since smoking, aging, and underlying diseases such as diabetes and dyslipidemia have been shown to reduce the quantity and quality of EPCs, the search for ways to improve EPC function will be an important issue in improving transplantation outcomes in the future.

DISCUSSION

In this paper, we have described the current state and issues of regenerative medicine in rheumatic diseases. Regenerative medicine is attracting so much attention that it is fundamentally changing the paradigm of treatment. It has the potential to become a new treatment option for patients with rheumatic diseases with poor prognoses. Especially in SSc, there is currently no effective drug therapy to prevent tissue fibrosis and restore lung function, so the application of regenerative medicine is strongly expected.

On the other hand, regenerative medicine still has issues to be solved not only in terms of technology but also in terms of society. Regenerative medicine is being actively debated in many countries, including its efficacy and ethical issues. Up to now, there has been a miscellaneous mixture of various regenerative medicine, from clinical research to medical treatment. Because regenerative medicine research is conducted in parallel with patient care, the various groups affected by the care inevitably participate in the debate. In addition to discussion based on avoiding harm, providing benefits, and respecting individual autonomy and justice, problems arise due to the lack of legal regulation of stem cell research and practice. The environment surrounding regenerative medicine research is changing rapidly, and the amount of expertise required is increasing daily. To fully ensure the rights and safety of subjects in clinical trials in the field of regenerative medicine, it is necessary to have clinical research coordinators who have expertise in scientific, ethical, and legal fields. How to realize regenerative medicine while cooperating with society will be an important issue essential for the future development of regenerative medicine.

AUTHOR CONTRIBUTIONS

RY conceived the review project and devised the manuscript. HN critically discussed the review. All the above-listed authors edited the manuscript.

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Highlights of Strategies Targeting Fibroblasts for Novel Therapies for Rheumatoid Arthritis

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Synovial fibroblasts of rheumatoid arthritis (RA) play a critical role in perpetuation of chronic inflammation by interaction with immune and inflammatory cells and in cartilage and bone invasion, but current therapies for RA are not directly targeted fibroblasts. Selectively fibroblast targeted therapy has been hampered because of lack of fibroblast specific molecular signature. Recent advancement in technology enabled us to gain insightful information concerning RA synovial fibroblast subpopulations and functions. Exploring fibroblast targeted therapies have been focused on inducing cell death via fibroblast associated proteins; interrupting fibroblast binding to matrix protein; blocking intercellular signaling between fibroblasts and endothelial cells; inhibiting fibroblast proliferation and invasion; promoting cell apoptosis and inducing cellular senescence, and modulating fibroblast glucose metabolism. Translation into clinical studies of these fibroblast targeted strategies is required for evaluation for their clinical application, in particular for combination therapy with current immune component targeted therapies. Here, several strategies of fibroblast targeted therapy are highlighted.

Keywords: synovial fibroblast, rheumatoid arthritis, fibroblast activation protein, metabolism, proliferation

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INTRODUCTION

Current therapies for rheumatoid arthritis (RA) have substantially improved the outcome of the disease and the quality of life of these patients. However, achieving and maintaining long-term remission is still challenging. Moreover, a significant proportion of RA patients do not adequately respond to current therapies (1). One of the reasons for the imperfect management of RA is that a critical cell type, fibroblasts, is not adequately targeted.

RA is primarily inflammation of the synovium, cartilage degradation, and bone erosion. In a normal joint, synovium is a thin loosely organized relatively acellular connective tissue without a basal membrane. Instead, synovium is bordered by a lining layer which comprises cells of monocyte in origin (type A synoviocytes) and resident fibroblasts (previously referred as fibroblast-like synoviocytes, type B synoviocytes) (2, 3). The hallmark of RA pathology is hyperplasia of the synovium. In RA, the synovium grows enormously into a mass-like tissue containing a large number of immune and inflammatory cells. In several aspects, RA synovium is considered an analog of a tumor (4). Thus, RA synovium displays neovasculature forming pannus and invades into adjacent cartilage and bone leading to joint destruction (5). The tumor like feature of RA synovium is largely contributed by fibroblasts (4, 6). RA fibroblasts proliferate and are resistant to apoptosis. They build stromal network which harbors immune and inflammatory cells. Moreover,

fibroblasts actively interact with immune and inflammatory cells leading to persistent inflammation of the synovium; support the formation of ectopic lymphoid follicles (7). In addition, fibroblasts are effector cells producing inflammatory cytokines participating inflammatory process and matrix metalloproteinase (MMP) and directly invading articular cartilage and subchondral bone. It is well-established that infiltration of immune and inflammatory cells in RA synovium is histologically heterogeneous between individual patients ranging from fully organized lymphoid structures to diffusely distributed lymphoid and myeloid cells throughout the synovium, and to scares of immune and inflammatory cells (pauci-immune). In contrast to the highly variable presence of immune and inflammatory cells, fibroblasts are invariably present in all pathotypes of synovitis (8). Thus, fibroblasts are indispensable in formation of RA synovitis and actively contribute to cartilage and bone destruction. Thereby, a therapeutic strategy directed at modulation of fibroblast function or ablation of fibroblasts has long been proposed (9–12). However, fibroblast targeted therapies have not yet been developed for clinical practice owing to poorly understood molecular mechanisms that drive synovial fibroblast behavior in RA. Recently, more insightful understanding of RA fibroblasts coupled by advanced technology enable us to explore fibroblast targeted therapies for RA. Readers are directed to excellent comprehensive reviews for molecular and cell biology of fibroblasts, their interplay with immune and inflammatory cells in the synovium, and discussion on restoration of synovial homeostasis in RA (12–16). In this article, I shall highlight several different strategies selectively targeting fibroblasts, which have been explored in preclinical arthritis models and/or in early phase of clinical investigations.

EXPANSION OF FIBROBLASTS IN RA SYNOVIUM

The number of fibroblasts in RA synovium increases substantially in both lining and sublining regions. The expansion of fibroblasts is the result of increased proliferation and decreased cell death (17–19). Earlier studies suggest that fibroblasts arise from local epithelial to mesenchymal transition and differentiation from pluripotent mesenchymal stem cells (20–22). There is little evidence indicating *in situ* proliferation of fibroblasts in the lining region. In contrast, recent studies have demonstrated proliferation of sublining fibroblasts (23–25). The gradient reduction of Thy1 (CD90) and augmentation of proteoglycan (PRG)-4 from sublining to lining fibroblasts suggest that divided fibroblasts migrate from sublining to lining region (24, 26). The origin of sublining fibroblasts is not clear. A recent study suggests that circulating preinflammatory mesenchymal (PRIME) cells, which bear hallmarks of synovial sublining fibroblasts, migrate into synovium during RA flare (27). It is conceivable that PRIME cells further differentiate into sublining fibroblasts upon interaction with endothelial cells (15, 26, 27). Several studies have shown that RA fibroblasts display aberrant apoptosis at several levels (28) and also probably due to increased autophagy (17, 19).

CELL SURFACE PROTEINS SERVE AS TARGETS FOR ABLATION OF FIBROBLASTS OR MODULATION OF FIBROBLAST FUNCTION

Several subpopulations of RA synovial fibroblasts have been described based on their distinct profiles of gene and protein expression. It is likely that these subpopulations of fibroblasts function differently, which are related to their locations in the compartments and interaction with other cells in the synovium (**Figure 1**) (23, 25, 29, 30). The strategy at ablation of fibroblasts has been hampered due to the lack of specific cell surface targets. Recently, several surface proteins have been described for identifying RA synovial fibroblast populations. Namely, CD55, podoplanin, and protein tyrosine phosphatase receptor sigma (PTPRS) are expressed by lining fibroblasts, while Thy1 (CD90), and CD248 are expressed on sublining fibroblasts (**Figure 1**).

The ideal cell surface targets should be expressed by all subpopulations of fibroblasts. CD34, cadherin-11, and fibroblast activation protein (FAP) can be detected in both lining and sublining fibroblasts (**Figure 1**) (23–25, 29–32). CD34, expressed by hematopoietic stem cells, is not a specific marker for fibroblasts. CDH11 is critical for lining layer formation and CDH11 deficient mice are resistant to arthritis induction (33); and has been explored as a therapeutic target. However, in a phase II clinical trial, an anti-CDH11 monoclonal antibody failed to show efficacy in RA patients with inadequate response to tumor necrosis factor (TNF) inhibitors (34).

Fibroblast Activation Protein

FAP is a type II transmembrane protein serving as a serine protease that cleaves the peptide bond between proline and other amino acids. This activity modifies various bioactive molecules (35, 36). FAP is exclusively expressed in fetal cells but not expressed in healthy adult tissue, except bone marrow derived mesenchymal stem cells and wounded tissues (37–40). FAP is best known for its presence in stromal fibroblasts found in over 90% of epithelial tumors (35, 36). FAP expression in tumor cells and stromal fibroblasts may involve tumor invasion since over-expression of FAP in epithelial cells or fibroblasts promoted cell invasion through extracellular matrix (41, 42). In RA synovium, FAP is highly expressed by fibroblasts in the lining layer and the sub-lining layers (4, 7, 29, 43, 44). The expression is highly specific to RA fibroblasts since FAP is expressed in low levels by osteoarthritic and none by normal fibroblasts (43). Similarly, we have demonstrated that FAP is highly expressed in the synovium of arthritic joints in murine models of RA, collagen-induced arthritis (CIA) and arthritis in SKG mice. The pattern of expression is similar to that in RA synovium (7, 43). Importantly, FAP is not expressed in synovium of normal mice. Moreover, levels of FAP expression in CIA joints is correlated with the severity of arthritis. Thus, scanning of FAP expression in the joint has been used to monitor disease activity in CIA (45). The function of FAP expressed by RA fibroblasts is not clear but may be related to the invasion of synovial pannus to cartilage and bone. This notion is supported by the following

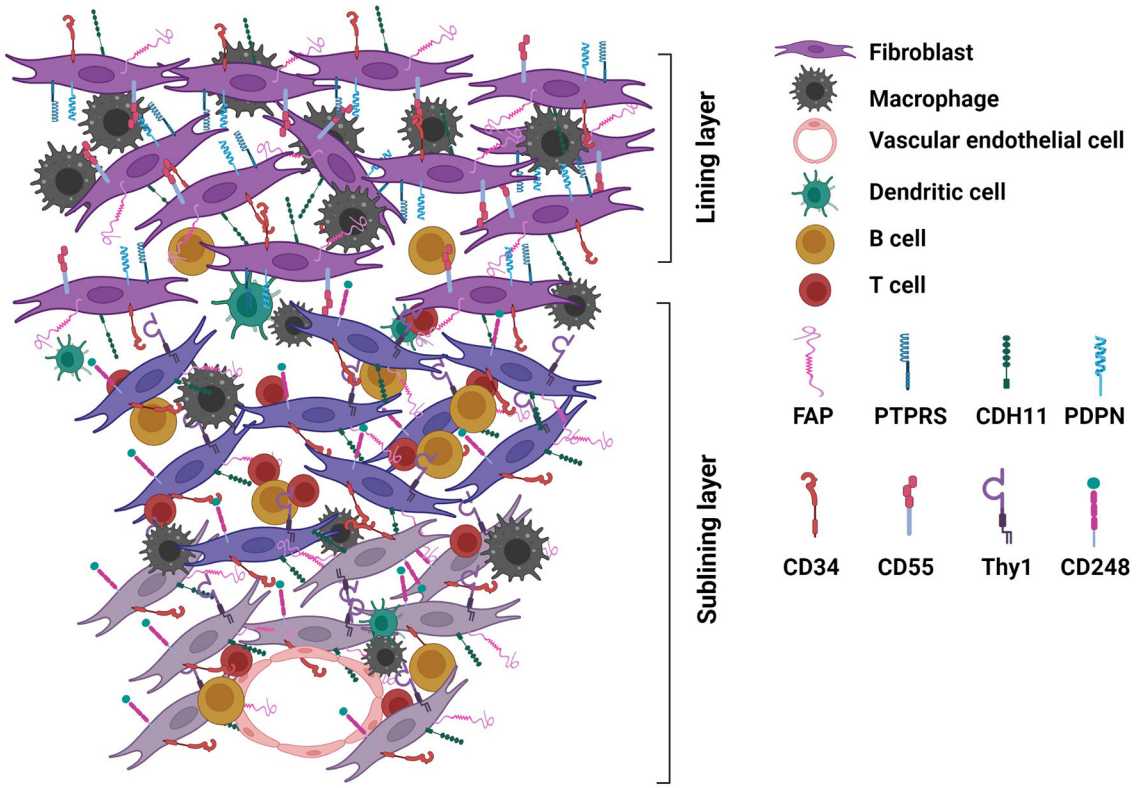

FIGURE 1 | Rheumatoid arthritis (RA) synovium highlighting fibroblasts with surface protein expression. Expansion of fibroblasts substantially contributes to the hyperplasia of RA synovium. Fibroblasts interplay with immune and inflammatory cells to perpetuate inflammation and invade cartilage and subchondral bone leading to joint destruction. Subpopulations of fibroblasts disperse in the lining and sublining layers of the synovium. Distinct profiles of surface proteins expressed by sublining and lining fibroblasts are highlighted here with relevance to fibroblast targeted therapies. Sublining fibroblasts express Thy1 (CD90) and CD248, while lining fibroblasts express CD55, podoplanin (PDPN), and protein tyrosine phosphatase receptor sigma (PTPRS). CD34, cadherin (CDH)-11 and fibroblast activation protein (FAP) are expressed by both sublining and lining fibroblasts [detailed summary of protein expression by different subpopulations of RA synovial fibroblasts is described in Nygaard and Firestein's review (12)].

experimental data. For instance, backcross of FAP gene deficient mice with human TNF transgenic mice lead to amelioration of cartilage damage (46). $FAP^+/Thy1^-$ fibroblasts primarily reside in the lining layer and produce MMP3, MMP9, and MMP13 which are involved in cartilage degradation. In addition, these lining fibroblasts also express CCL9 and TNFSF11, both potently induce osteoclast activity. They also express high levels of receptor activator of nuclear factor kB receptor ligand (RANKL). Whereas, FAP^+/Thy^+ fibroblasts mainly reside in the sublining layers are mediating inflammation. These distinct activities of the two subpopulation of fibroblasts were elegantly demonstrated by transfer studies (29). Furthermore, depletion of FAP expressing fibroblasts in serum transfer arthritis model reduced arthritis severity (29). Importantly, FAP gene knockout mice develop normally and have no clinical disease phenotype, suggesting that targeting FAP will be less likely to cause fatal adverse effects (47).

Several strategies have been explored in cancer immunotherapy by targeting FAP in tumor models, which can be applied in treating RA. These include monoclonal antibodies, DNA vaccines and chimeric antigen receptor (CAR) T cells against FAP (**Figure 2A**).

Unfortunately, phase I/II clinical trials with an anti-FAP monoclonal antibody in cancer treatment did not show meaningful clinical efficacy probably due to the fact that the non-toxic unconjugated monoclonal antibody did not induce cell death (48). Recently, Dorst et al. (49) conjugated a near infrared dye, IRDye700DX to a monoclonal antibody (28H1) to FAP and demonstrated that *in vitro* 28H1-700DX was capable of killing FAP expressed fibroblast cell line (3T3) and primary synovial fibroblasts from synovial biopsy of RA patients (50). Furthermore, 28H1-700DX had moderate effect on development of CIA presumably by inducing death of FAP expressing synovial fibroblasts (49). These data indicate the feasibility of anti-FAP monoclonal antibody based therapy for RA is still a viable approach provided that the anti-FAP monoclonal antibody is able to ablate fibroblasts.

DNA vaccines and CAR T cells have been demonstrated to be efficacious for immunotherapy in cancer models and cardiac fibrosis (51–53). Employment of vaccines and CAR T cells to treat RA is distinctive from current RA therapies. Since RA is a chronic disease, it is highly desirable for RA patients to have a treatment which the efficacy will last for a long-term.

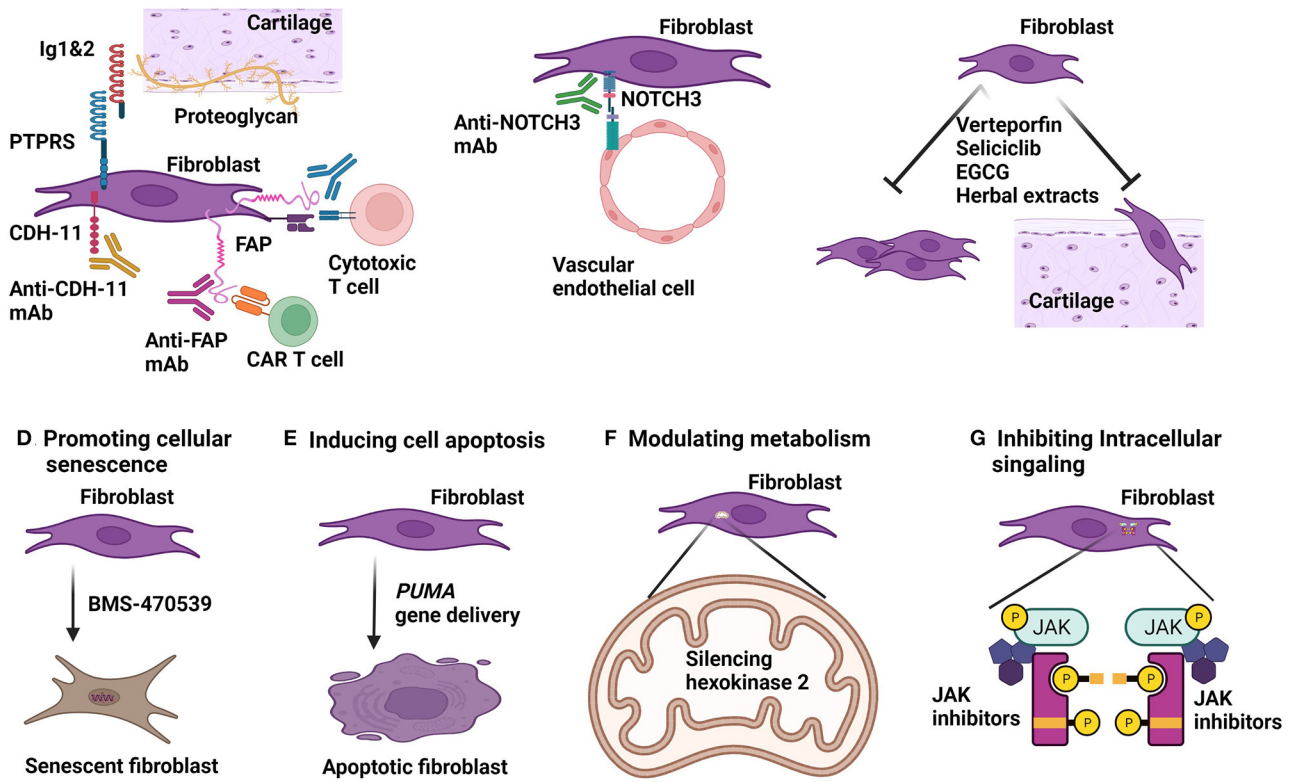

FIGURE 2 | Strategies for fibroblast targeted therapy. **(A)** Cell surface expressed proteins can be targeted in various ways. Therapeutic monoclonal antibodies (mAb) to cadherine-11 (CDH-11) and fibroblast activation protein (FAP) have been developed. FAP can also be targeted by chimeric antigen receptor (CAR) T cells and by vaccination which will provoke antibodies and cytotoxic T cells against FAP *in vivo*. Immunoglobulin like domain 1&2 (Ig1&2) acts as a decoy receptor blocks protein tyrosine phosphatase receptor type S (PTPRS) binding to proteoglycan. **(B)** mAb to neurogenic locus notch homolog protein 3 (NOTCH3) can block interaction between fibroblasts and endothelial cells. **(C)** Several approaches to inhibition of fibroblast proliferation and migration have been investigated. Transcriptional co-activators, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are active in RA fibroblasts. Verteporfin blocks YAP/TAZ binding to transcriptional enhanced associate domains (TEAD) and has anti-arthritic effect in arthritis models. Seliciclib blocking RA fibroblast proliferation and has been in a clinical trial for treating RA. Green tea extract, epigallocatechin-3-gallate (EGCG) and several Chinese herbal extracts are shown to inhibit RA synovial fibroblast proliferation, migration and invasion *in vitro* and anti-arthritic effect in animal models of arthritis. **(D)** Activation of a G-protein coupled receptor, melanocortin type 1 receptor (MC1R) by a selective agonist, BMS-470539 can induce senescence and diminish activity of RA fibroblasts. **(E)** Delivery of pro-apoptotic gene, *PUMA* (p53 upregulated modulator of apoptosis) induced RA synovial fibroblast cell death and has anti-arthritic effect in arthritis models. **(F)** Glucose metabolism of RA synovial fibroblasts are high active. Silencing hexokinase 2, a glycolytic enzyme resulted in reduction of migration and invasion of RA fibroblasts *in vitro*. **(G)** Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway is active in RA synovial fibroblasts. Fibroblast-selective JAK inhibitors are required to inhibit JAK-STAT mediated cytokine signaling for RA synovial fibroblasts.

Vaccination fulfills this requirement. CAR T cells can become memory effector cells (54) and provide continuously surveillance to the joint and generate specific response to FAP to prevent fibroblast expansion. Furthermore, targeted therapies against fibroblasts have the potential to modify disease persistence as opposed to simply inhibiting inflammation. Both vaccination and CAR T cells potentially provide memory immunity against activated fibroblasts by attacking the activation molecule, FAP. These therapies therefore may offer a one-time treatment for a long-term remission. The vaccination approach can also be applied in prevention of RA onset in those at risk population.

Vaccination against FAP has been extensively studied for cancer immunotherapy in animal models. Various strategies have been used. Plasmid cDNA encoding FAP has successfully provoked cellular immune response to FAP and benefit to the host in reducing tumor burden. Since RA is a chronic disease with persistent synovitis which is presumably the result from sustained stimuli. Therefore, a long lasting immunity against FAP will be required to suppress arthritis. Plasmid cDNA injection combined with electroporation which has been successfully applied in tumor models (51) and is being tested in human clinical trials for human papillomaviruses (HPV) (Phase 2b) (55) and Zika virus (Phase 1) (56). The anti-HPV DNA vaccine showed efficacy in treating cervical intraepithelial neoplasia (55); and anti-Zika DNA vaccine elicited anti-Zika virus immune responses (56). It is expected that FAP DNA vaccine will

elicit both antibody and T cell immune responses to FAP and arthritis suppression.

Treating solid tumors with FAP CAR T cells alone or in combination with tumor specific antigens have been investigated with success in multiple preclinical tumor models (52, 57, 58). It is expected that CAR T cells against FAP will also display therapeutic effects in arthritis models. Considering that CAR T cells may generate long-lasting memory immune response and one time treatment is needed will justify its application.

Since FAP is an endogenous protein, toxicity or off target effects upon depletion of FAP expressing cells has been a concern. However, data from preclinical studies support this approach is a viable safe therapy. For example, mice with genetic ablation of FAP grow normally without pathological phenotypes (47). Many studies reported successful inhibition of tumor growth but without severe clinical toxicity or impaired wound healing (51, 52, 58–63). In contrast, a recent study with genetic ablation of FAP in BM-MSC showed severe toxicity with anemia and weight loss (59). Explanation for the toxicity include direct suppression of hemopoietic cells and depletion of fat tissue. The findings in this study is in contrast to those with globally targeting FAP expressing cells. These include recently published studies using DNA vaccines (51) and CAR T cells against FAP (52). Both studies have been successfully used in tumor models with efficacy in inhibition of tumor growth without severe toxicity. In particular, there were no cachexia or anemia observed in animals whose FAP expressing cells were depleted. Since FAP is not expressed by normal fibroblasts, depletion of FAP expressing cells does not affect normal fibroblasts. Data from the most recent study confirmed that depletion of FAP expressing fibroblasts in an arthritis model was not toxic (29). Thus, FAP expressing cells were depleted by injection of diphtheria toxin during arthritis in transgenic FAP luciferase diphtheria toxin receptor reporter mice. All these observations support the feasibility and safety for a treatment of arthritis by depleting FAP expressing fibroblasts. The first in human Phase 1 clinical trial with CAR T cells against FAP in mesothelioma patients is reported to be feasible and safe (64). Needless to say, further human studies are required to assess the usage of FAP targeted CAR T cell therapy for non-malignant conditions such as RA.

Protein Tyrosine Phosphatase Receptor Sigma

Another transmembrane protein, PTPRS is highly enriched in RA and experimental arthritis synovial lining fibroblasts (**Figure 1**) (31, 32). PTPRS acts like a receptor or ligand binding via N-terminal extracellular immunoglobulin-like domains 1 and 2 (Ig1&2) to extracellular matrix protein, proteoglycan (PG) of various types. Ig1&2 may bind to heparin sulfate (HS) or chondroitin sulfate (CS) glycosaminoglycan (GAG) moieties of PG (65). HS-containing PG competes with CS-containing PG in binding to PTPRS, a mechanism is termed "PG switch" (65). Synovial fibroblast attachment to cartilage is an important process for invasion of cartilage during inflammatory arthritis (66). This attachment is PG dependent and mediated by HS moieties and can be inhibited by exogenous cartilage derived CS (31). This suggests that manipulation of PG switch in arthritic joint can be therapeutic. Furthermore, soluble recombinant Ig1&2 can act as a decoy receptor to inhibit PTPRS expressed by fibroblasts binding to PG, thereby be used to treat arthritis (**Figure 2A**). Indeed, Ig1&2 decoy receptor suppressed KBxN serum transfer induced arthritis and chronic arthritis in KBxN mice (31). Interestingly, a fusion protein, Fc-Ig1&2 decoy receptor treatment can be combined with TNF inhibition and synergistic therapeutic effect is achieved in KBxN serum transfer arthritis and CIA (32). PTPRS is highly expressed in human RA synovial fibroblasts. Fc-IgI&2 inhibits RA synovial fibroblasts motility *in vitro* (32). All these results suggest that manipulation of PG switch between PTPRS and PG can be potentially applied for treatment of human RA and may be combined with TNF inhibition.

PG switch is in operation in central nervous system. PTPRS is expressed by neurons. Binding of PTPRS to HS-containing PG causes oligomerization and functionally inactivation for axonal extension. In contrast, binding of PTPRS to CS-containing PG inhibits axonal growth (65). Moreover, inhibition of PTPRS can induce neuronal regeneration in spinal cord contusion models (67, 68). These results suggest that strategies in inhibition of PTPRS is therapeutic for arthritis and in favor of neuronal regeneration. We hope that the same beneficial effects of PTPRS inhibition will also be achieved in treating human RA.

INTERRUPTING INTERCELLULAR SIGNALING FOR MODULATION OF FIBROBLASTS

In RA synovium, the expanded fibroblasts in the lining and sublining layers reside in different anatomical regions and execute related but different activities (29), and phenotypically are distinct subpopulations. However, they are likely derived from same origin. Perivascular fibroblasts receive signals from vascular endothelial cells to proliferate and differentiate and migrate to lining layer. This process is evident by gradient increase of PRG4 but decrease of Thy1 expression from sublining to lining fibroblasts (24). Among the signals conduced between endothelial cells and fibroblasts, NOTCH signaling, particularly NOTCH3 is critical. NOTCH3 is highly expressed by perivascular fibroblasts. In an organoid culture system, silencing NOTCH3 gene expression by siRNA diminished endothelium induced fibroblast expansion and gradient cell surface Thy1 and PRG4 expression. The critical role of NOTCH signaling has been tested in animal models of arthritis. In a rat CIA model, a NOTCH1 and NOTCH3 inhibitor (LY411575) showed therapeutic effect of arthritis, presumably by inducing fibroblast death since LY411575 is able to induce death of human synovium derived fibroblast cell line, MH7A *in vitro* (69). Using monoclonal antibodies, Wei et al. (24) demonstrated that anti-NOTCH3 monoclonal antibody has profound therapeutic effect in arthritis compared with moderate effect by monoclonal antibody to NOTCH1 (**Figure 2B**). Furthermore, NOTCH3 knockout mice had diminished arthritis induced by KBxN serum transfer compared with wild type animals (24). NOTCH3 gene deleted

mice develop a normal joint structure suggests that NOTCH3 targeted therapy is safe for treating arthritis (24, 26). Further development for RA therapy by targeting NOTCH3 signaling is warrant.

TARGETING SYNOVIAL FIBROBLAST PROLIFERATION AND INVASION

Several modalities with various mechanisms of action are able to inhibit proliferation, migration and/or invasion of RA synovial fibroblasts (**Figure 2C**) and have been investigated for development of fibroblast targeted therapy.

Yes-Associated Protein and Transcriptional Co-activator With PDZ-binding Motif

Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) are transcriptional co-activators involving in regulation of cell growth and differentiation during development and neoplastic progression (70). YAP and TAZ share structure similarity and have overlapped functions; both exert their transcriptional activity via translocation to the nucleus and by interaction with transcriptional enhanced associate domains (TEAD). YAP/TAZ transcriptional activity is enhanced in RA fibroblasts to promote their proliferation and invasive behavior (71, 72). *In vitro*, blocking YAP/TAZ activity using verteporfin, which inhibits YAP/TAZ binding to TEAD, resulted in reduced RA fibroblast resistance to apoptosis, diminished proliferation, less invasion, and poor inflammatory response (72). Moreover, in organoid culture system, verteporfin was able to prevent RA fibroblasts to form synovial lining layer and interrupt already formed lining layer. This effect was the result of YAP/TAZ mediated c-Jun nuclei translocation that was suppressed by verteporfin. Further, in an adjuvant induced arthritis (AIA) model, administered before arthritis onset, verteporfin reduced arthritis severity although arthritis was not prevented. After onset of arthritis, verteporfin was able to block further progression of arthritis (71). In another study, YAP was found to form a complex with PTPN14 to recruit SMAD3 to nucleus of RA synovial fibroblasts to induce the aggressive behavior. Verteporfin decreased RA synovial fibroblast invasion into cartilage *in vivo* in a severe combined immunodeficiency mouse model and reduced severity of KBxN serum transferred arthritis (71). All these data indicate that YAP/TAZ contributes to the invasive behavior of RA synovial fibroblasts and interrupting YAP/TAZ transcriptional activity is a viable strategy to explore as an alternative therapy for RA.

Seliciclib—Cell Cycle Dependent Kinase Inhibitor

Cell cycle dependent kinase (CDK) inhibitor, seliciclib is under development for cancer therapy (73). Seliciclib suppresses synovial fibroblast proliferation by inhibiting CDK2 and induction of endogenous CDK inhibitor p21 which was shown to be down regulated in synovial fibroblasts of RA patients (74, 75). In addition, independent of cell cycle inhibition, seliciclib was shown to inhibit expression of collagen, fibronectin and connective tissue growth factor in normal and scleroderma fibroblasts (76). In a KBxN serum transfer arthritis model, injection of seliciclib significantly reduced the severity of arthritis (77) although the therapeutic effect of seliciclib was not directly attributed to inhibition of fibroblasts. Based on these findings, a phase Ib clinical trial was conducted in 15 RA patients who display active disease despite treatment with TNFi either as monotherapy or in combination with other disease modifying anti-rheumatic drugs (DMARD) (78). The trial was designed to assess safety of seliciclib in treating RA patients. The maximum tolerable dose is 400 mg/day; the safety profile is acceptable for future efficacy trial. At 4 weeks, 9 patients showed reduction of DAS28-CRP score although these may represent regression to the mean. Nevertheless, this finding indicates further evaluation of seliciclib for RA treatment, especially in combination with other DMARDs including TNFi is warrant. Seliciclib is not myelossuppressive, therefore, exceeding immunosuppression is not expected in combination of seliciclib with other DMARDs.

Epigallocatechin-3-Gallate

Epigallocatechin-3-gallate (EGCG), a compound derived from green tea, displays potent antioxidant, anti-inflammatory, and antioncogenic activity (79); and has been shown to be able to ameliorate arthritis in animal models (80, 81). Effects of EGCG on RA synovial fibroblasts are multifold. In the *in vitro* culture system, EGCG does not directly cause cytotoxicity of RA synovial fibroblasts, but promotes apoptosis in TNF sensitized cells by blocking myeloid cell leukemia 1 expression (82); inhibits IL-1β induced chemokine production and MMP2 activation (83); and inhibits IL-6 synthesis and suppresses IL-6 trans-signaling by inducing production of soluble gp130 production (80). It is required to determine whether the *in vivo* anti-arthritic effect of EGCG takes place by selectively targeting on synovial fibroblasts but not affecting other effector cells in arthritis. It has been shown that in IL-1 receptor antagonist knock mouse arthritis and CIA models, EGCG attenuates arthritis by inhibiting STAT3 and hypoxia induced factor-α which leads to reduction of Th17 cells (84, 85). The effects of EGCG on other cell types indicates its wide spectrum of antiinflammatory effect and may not be a viable candidate for fibroblast targeted therapy.

Chinese Herbal Extracts

Similarly, extracts from Chinese herbal drugs such as kirenol (86), piperlongumine (87) and 3,3′-diidolylmethane (88) have been shown *in vitro* to inhibit RA synovial fibroblast proliferation, migration, and invasion. In addition, they were able to suppress MMP production by RA fibroblasts. Moreover, kirenol and 3,3′-diidolylmethane are able to attenuate CIA and AIA, respectively (86, 88). Further investigations are required to delineate whether the effects of these drugs are specific to fibroblasts.

PROMOTION OF CELLULAR SENESCENCE OF FIBROBLASTS

Promotion of cellular senescence has been proposed and actively explored as an approach to cancer therapy (89, 90). RA synovial

fibroblasts display some features of tumor cells (4), thereby, pro-senescence in these fibroblasts is potentially therapeutic for RA (**Figure 2D**). Indeed, Montero-Melendez et al. (91) demonstrated that induction of synovial fibroblast senescence via activation of a G-protein coupled receptor, melanocortin type 1 receptor (MC1R) can ameliorate an experimental arthritis. MC1R is highly expressed by RA fibroblasts. Activation of MC1R using a selective agonist, BMS-470539, but not a non-selective agonist, induced senescence in RA fibroblasts. The specific role of MC1R in this senescence was further confirmed in primary mouse synovial fibroblasts that *MC1R* knockout abrogate the effect of BMS-470539. Furthermore, this MC1R activation induced fibroblast senescence involves phosphorylation of extracellular signal-regulated protein kinase (ERK) 1/2, a mitogen-activated protein kinase (MAPK) family protein, which have been shown to promote cellular senescence (92). Importantly, BMS-470539 was able to suppress KBxN serum transfer induced arthritis in mice and the therapeutic effect was associated with *in vivo* synovial fibroblast senescence. The therapeutic effect of BMS-470539 was countered by senolytic drugs (91).

These results are encouraging although further confirmatory evaluation in chronic arthritis models would be required for long-term efficacy and possible adverse effects associated with pro-senescence. Senescence of joint cells, especially chondrocytes is increased in osteoarthritis (OA) (93). It is to be determined if therapy with pro-senescence in fibroblasts and off-targeted senescence of other joint cell types will result in OA of the joint. Another concern associated with pro-senescence as a therapy is the removal of senescent cells from the tissue. Insufficient elimination of the senescent cells after induction of pro-senescence might be detrimental and senolysis may be required. Interestingly, local clearance of senescent cells is beneficial in post-traumatic OA model (94).

INDUCTION OF FIBROBLAST APOPTOSIS

RA synovial fibroblasts show reduced rate of apoptosis. Thereby, inducing cell death is an attractive approach to ablate synovial fibroblasts for RA therapy (**Figure 2E**).

p53 Upregulated Modulator of Apoptosis

Somatic mutation of tumor suppressor gene, *TP53* (encoding a protein commonly called p53) is one of the important mechanisms responsible for insufficient apoptosis and invasiveness of RA fibroblasts (95, 96). Thereby, promoting apoptosis of fibroblasts is a plausible strategy for RA therapy. Along this line, Firestein and colleagues first demonstrated that *PUMA* (p53 upregulated modulator of apoptosis) gene expression is deficient in RA fibroblasts (97); subsequently, adenovirus vector transfection of *PUMA* resulted in rapid apoptosis of fibroblasts with the activation of caspase 3 (97). Interestingly, *PUMA* induced apoptosis is independent of p53 expression (98), which is of significance in practice for developing a therapy since induction of *PUMA* expression alone without presence of active p53 is sufficient to induce death of these fibroblasts in the synovium. However, gene therapy with *PUMA* had been hampered by poor gene delivery efficiency in fibroblasts until recently. This problem was circumvented by Hong et al. (99) by conjugating human adenovirus type 5 (HAdV5) to a baculovirus vector expressing the Coxsackie-adenovirus receptor (CAR) on its envelope. The modified BV^{CAR}-HAdV5 vector efficiently delivered *PUMA* gene (BV^{CAR}-HAdV5-PUMA) into RA synovial fibroblasts and induced rapid cell death *in vitro*. Furthermore, in a rat AIA model, single intraarticular injection of BV^{CAR}-HAdV5-PUMA significantly reduced inflammation, improved joint function, and decreased joint erosion and bone loss. HAdV5 is a biological safe virus vector for gene therapy, but the intraarticular delivery limited its further development as a viable therapeutic strategy since RA patients require systemic treatment.

Cadmium

Metal element, cadmium has p53-dependent pro-apoptotic properties (100) and has been tested for treating arthritis in animal models (101). Cadmium was able to induce apoptotic cell death of synovial fibroblasts isolated from patients with RA. Intraarticular injection of cadmium in rats with AIA can suppress inflammation. Although this proof of concept study demonstrated the anti-arthritic effect of cadmium, several issues including toxicity, association of cadmium with an increased risk of RA (102, 103), and local administration will limit its use as a therapeutic for RA.

INHIBITION OF GLUCOSE METABOLISM OF FIBROBLASTS

The tumor-like behaviors of RA fibroblasts such as activation, migration and invasion are associated with increased cell metabolism. There is ample evidence to indicate that glucose metabolism plays an important role. Therefore, interruption of glucose metabolism of fibroblasts is a plausible approach to novel therapy of RA. As has been demonstrated in several murine models of RA, inhibition of glycolysis can significantly reduce severity of arthritis (104, 105). However, global inhibition of glycolysis is not desirable as a therapy. Fibroblast specific inhibitor of glucose metabolism is required. Among all the glycolytic enzymes involved, hexokinase 2 (HK2) may be a relatively selective target (**Figure 2F**). HK2 is an inducible isoform that is selectively highly expressed in skeletal and cardiac muscles and adipose tissue (106), and is highly expressed in RA but little in OA synovial tissue (107). Silencing HK2 resulted in reduction of migration and invasion of RA fibroblasts *in vitro*. Ablation of HK2 significantly reduced severity of arthritis and bone and cartilage damage in KBxN serum transfer induced arthritis (107).

Currently, a HK2 specific inhibitor is not available, but a glucose analog, 2-deoxy-D-glucose (2-DG) has been extensively evaluated as an agent for inducing cancer cell death and cancer therapy (108). Administration of 2-DG reduced the severity of spontaneous arthritis in KBxN mice. However, the therapeutic effect of 2-DG was attributed to inhibition of glucose metabolism

in T follicular helper (Tfh) cells. The effect of 2-DG on fibroblast activity remains to be possible, but unfortunately this was not investigated in this work (109). This result on T cells is in consistence with the previous findings that loss of HK2 mildly reduced colitis in interleukin-10 deficient mice and ovalbumin induced airway inflammation (110). Furthermore, HK2 is not essentially required for T cell glucose metabolism *in vitro* and loss of HK2 did not impair clearance of lymphocytic choriomeningitis virus infection, suggesting inhibiting HK2 activity would have less immune compromise (110). Further studies are required to clarify the targeted cell types of 2-DG in RA models and a fibroblast specific HK2 inhibition strategy is required to develop.

JAK INHIBITORS FOR FIBROBLAST TARGETED THERAPY

Janus kinase (JAK) inhibitors are approved therapy for RA. JAK-signal transducer and activator of transcription (STAT) pathway is active in RA synovial fibroblasts in response to cytokine stimulation (111). As expected, JAK inhibitors are able to block cytokine mediated inflammatory production by fibroblasts; these include stimulation by cytokines such as oncostatin M, IL-6, TNF, IL-1, and interferon-γ (111, 112).

Current JAK inhibitors are not fibroblast specific. Among the JAK inhibitors, peficitinib was able to suppress synovial fibroblast migration *in vitro* and may induce fibroblast apoptosis suggesting that peficitinib may have advantage over other JAK inhibitors on targeting fibroblasts (**Figure 2G**) (113, 114). Since high dose of peficitinib is tolerated, it may in particular be useful for treating RA patients with pauci-immune pathotype which is poorly responsive to conventional DMARDs or TNF inhibitors (115). Selective JAK inhibitors for JAK-STAT pathway in fibroblasts are to be uncovered for this purpose. On the other hand, synovial fibroblasts are major source of IL-6 production in RA joint (25, 116). Moreover, IL-6 acts in an autocrine amplification mechanism on activation of fibroblasts (116). Therefore, JAK inhibitors with profound activity to block IL-6 signaling may have profound impact on fibroblast activation in RA.

TABLE 1 | Agents for fibroblast targeted therapy for RA*.

Agent	Target and mechanism of action	Developing stage	References
	Cell surface protein as target		
Monoclonal antibodies	CDH-11: inhibit formation of synovial lining layer	Phase II (ineffective)	(34)
Monoclonal antibodies	FAP: ablation of fibroblasts	Pre-clinical	(49, 50)
Fc-Ig1&2	PTPRS: decoy receptor to inhibit PTPRS binding to proteoglycan and block fibroblast invasion	Pre-clinical	(31, 32)
	Interruption of intercellular signaling		
Monoclonal antibodies	NOTCH3: blocking NOTCH3 signaling to inhibit fibroblast differentiation	Pre-clinical	(24)
	Inhibition of fibroblast proliferation and invasion		
Verteporfin	YAP/TAZ: blocks YAP/TAZ binding to TEAD to inhibit fibroblast proliferation and invasion	Pre-clinical	(71, 72)
Seliciclib	CDK2: blocks kinase activity to inhibit fibroblast proliferation	Phase I	(78)
EGCG	Pleotropic: anti-inflammatory, anti-oxidant and anti-oncogenic. Inhibits fibroblast proliferation	Pre-clinical	(80, 81)
Chinese herbal extracts	Likely pleotropic: inhibit fibroblast proliferation and invasion	Pre-clinical	(86, 88)
	Induction of cellular senescence		
BMS-470539	MC1R: agonist, activates MC1R to induce fibroblast senescence	Pre-clinical	(91)
	Promotion of apoptosis		
BV^{CAR}-HAdV5-PUMA	PUMA: virus mediated gene delivery to induce fibroblast apoptosis	Pre-clinical	(99)
Cadmium	Metal element: induction of fibroblast apoptosis	Pre-clinical	(101)
	Inhibition of glucose metabolism		
2-deoxy-D-glucose (2-DG)	HK2: non-HK2 selective inhibitor, inhibit fibroblast glucose metabolism	Pre-clinical	(109)
	JAK inhibitor and anti-fibrotic drugs		
Peficitinib and others	JAK: inhibits JAK activity, non-selective. Approved clinical treatment of RA, but fibroblast selective JAK inhibitors are to uncover		(113, 114)
Pirfenidone	Anti-fibrotic: approved for treating idiopathic pulmonary fibrosis	Pre-clinical (repurpose for treating RA)	(118)
Nintedanib	Anti-fibrotic: approved for treating idiopathic pulmonary fibrosis	Pre-clinical (repurpose for treating RA)	(119)

**RA, rheumatoid arthritis; CDH-11, cadherin-11; FAP, fibroblast activation protein; Fc-Ig1&2, IgG Fc-immunoglobulin-like domains 1&2 fusion protein; PTPRS, protein tyrosine phosphatase receptor sigma; NOTCH3, neurogenic locus notch homolog protein 3; YAP/TAZ, Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ); TEAD, transcriptional enhanced associate domains; CDK2, cell cycle dependent kinase 2; EGCG, epigallocatechin-3-gallate; MC1R, melanocortin type 1 receptor; PUMA, p53 upregulated modulator of apoptosis; BV^{CAR}-HAdV5-PUMA, human adenovirus type 5 (HAdV5) to a baculovirus vector expressing the Coxsackie-adenovirus receptor (CAR); HK2, hexokinase 2; JAK, Janus kinase.*

ANTI-FIBROTIC DRUGS

Pirfenidone [5-methyl-1-phenyl-2-(1H)-pyridone], one of the heterocycle pyridones, is approved for treating idiopathic pulmonary fibrosis. Pirfenidone was first developed for anti-pyretic and analgesic use, but was found to have anti-fibrotic effect. Pirfenidone has pleiotropic effects including inhibition of transforming growth factor (TGF)-β-mediated proliferation of fibroblasts. In cultured synovial fibroblasts, pirfenidone can reduce the expression of intercellular adhesion molecule-1. Pirfenidone can also suppress TGF-β induced collagen type I production and inhibit myofibroblasts activity to reduce extracellular matrix deposition (117). In a rat CIA model, pirfenidone was shown to ameliorate arthritis and reduction of MMP3 and vascular endothelial growth factor (VEGF) expression in the joint (118). In Simian Vacuolating Virus 40 large T antigen transformed human synovial fibroblast cell line, MH7A cells, pirfenidone significantly reduced TNF-induced production of inflammatory cytokines, VEGF, and MMPs. Moreover, pirfenidone significantly inhibited VEGF by vascular endothelial cell line, EA.hy926 cells (118). Further studies are required to delineate whether these effects of pirfenidone fibroblast selective.

Another anti-fibrotic drug, nintedanib, a potent tyrosin kinase inhibitor that inhibits growth factor stimulated migration and proliferation of fibroblasts and TGF-β-induced transformation to myofibroblasts. In a zymosan-induced arthritis and pulmonary fibrosis model in SKG mice, treatment at early stage of disease, nintedanib was able to significantly reduce severity of arthritis but has no effect on progression of lung fibrosis. In contrast, treatment started when pulmonary fibrosis established, nintedanib was able to reduce pulmonary fibrosis but had no effect on established arthritis (119). Nintedanib is approved for treating idiopathic pulmonary fibrosis. There are case reports in the literature that nintedanib has been tried in RA related interstitial lung disease and showed benefit (120). It would be interesting to investigate further how ninetedanib will affect synovial fibroblasts in RA patients and whether ninetedanib will show anti-arthritic effect.

CONCLUDING REMARKS

RA synovial fibroblasts are valid therapeutic target candidates and current strategies for intervention have been explored (**Table 1** and **Figure 2**). Especially, fibroblasts are not professional immune cells for host defense although they display features of inflammatory cells and can present self-antigens during the disease process of RA. Therapies directed at suppression of the function or ablation of synovial fibroblasts will be less likely to cause immunosuppression. Therefore, development of fibroblast targeted therapy will be particularly practical for combination therapy with currently available immune component directed therapies. Another concern with regard to fibroblast ablation therapy is impairment of wound healing since fibroblasts are critical for tissue repair. However, in preclinical tumor models, ablation of fibroblasts did not negatively affect wound healing although these need to be further evaluated in human studies (51, 52, 59).

In addition to the strategies discussed above, mitogen-activated protein kinase (MAPK) are highly activated in RA synovial fibroblasts. However, targeting MAPK pathway for therapy has not be successful in clinical trials (discussed in details by Nygaard and Firestein (12). Also, targeting the imprinted signature of RA synovial fibroblasts is another attractive approach. For example, modulation of histone-modifying enzymes may lead to remodeling and restoration of the homeostasis of RA synovial fibroblasts [reviewed by (12)].

Clinically, RA patients with pauci-immune synovial pathotypes respond poorly to current DMARDs which are mainly directed to immune suppression. Presumably fibroblasts are the predominant effector cell types mediating the disease process in these patients (8, 115). This RA subpopulation will better serve for testing efficacy of fibroblast targeted therapies in clinical studies. Peficitinib, one of the newer JAK inhibitors was shown to preferentially act on JAK-STAT pathway in RA synovial fibroblasts and hence would be a good candidate to try on this RA subpopulation (113, 114).

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The author confirms being the sole contributor of this work and has approved it for publication.

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IL-13 and IL-33 Serum Levels Are Increased in Systemic Sclerosis Patients With Interstitial Lung Disease

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Objective: Systemic sclerosis (SSc) mortality is extremely variable in its internal organ involvement. Pulmonary fibrosis occurs in up to 30% of the cases. Animal models provide evidence that IL-33 is able to induce both cutaneous and pulmonary fibrosis via increased IL-13 and in SSc patients the levels of IL-33 correlate with skin fibrosis. Our aim was to test whether both IL-33 and IL-13 are higher in patients with diffuse SSc and interstitial lung disease (SSc-ILD) compared to SSc patients without ILD and healthy controls.

Methods: Serum levels of IL-13 and IL-33 were measured in 30 SSc patients with diffuse disease and 30 healthy controls by enzyme-linked immunosorbent assay. The extent of pulmonary fibrosis was assessed according to HRCT Warrick score. Pulmonary function tests included lung diffusion capacity for carbon monoxide, forced vital capacity and total lung capacity.

Results: Both IL-13 and IL-33 levels were increased in SSc patients compared to controls and significantly associated each other. DLco, FVC and TLC scores were inversely associated with IL-33 and IL-13 levels. Both IL-33 and IL-13 levels were significantly associated with the Warrick severity score and higher in the group of SSc patients with reduced pulmonary function compared to SSc patients with normal pulmonary function tests.

Conclusion: The IL-13/IL-33 axis needs to be further explored in longitudinal studies of SSc-ILD patients to assess its validity as a biomarker and future treatment target, as does downstream mediator ST2.

Keywords: systemic sclerosis, interstitial lung disease, IL-33, IL-13, interleukins

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INTRODUCTION

Systemic sclerosis (SSc) is a connective tissue disease characterized by the triad of microvascular injury, immunologic activation and fibrosis (1). The clinical phenotype of the disease differs widely. It is therefore crucial to apply strict patient selection criteria to identify effective treatments for life-threatening organ complications, thereby addressing one of the main clinical unmet need in SSc research (2). In up to 60% of SSc cases mortality is due to pulmonary involvement and half of these cases are related to the development of pulmonary fibrosis, with the other half due to pulmonary hypertension (3).

Although the pathogenesis of SSc needs to be clarified, previous studies confirm that some cytokines and growth factors influence fibrosis progression in SSc by stimulating the activation of fibroblasts, impairing endothelial cells activity, and altering immune system function (4, 5). We focused our attention in particular to IL-13 and IL-33, in light of the growing body of recent evidence suggesting a possible role for these cytokines in fibrogenesis.

IL-33 is one of the most recently discovered member of the IL-1 cytokine family (6–9), while IL-13 is a prototypical Th2 cytokine (10–12). Recent evidence show that the Th2 cytokines IL-4 and IL-13 are higher in SSc patients and promote fibrotic responses (12–14).

Animal models studies demonstrated that IL-33 is able to induce fibrosis via increased IL-13 both in cutaneous (15) and pulmonary (16) fibrosis. In addition, the level of IL-33 is correlated with the extent of skin fibrosis in SSc patients–being higher in patients with the diffuse form compared to those with limited SSc—as well as with forced vital capacity (FVC) scores (17–19). Furthermore, the polymorphism of IL-33 gene rs7044343 is associated with SSc susceptibility and pulmonary manifestations in different populations (20, 21).

Indeed, IL-33 and its receptor ST2 are abnormally expressed in SSc and it has been postulated that in early SSc, IL-33 could be mobilized from areas of vascular damage to promote fibrosis in target organ through ST2 (18, 22) and the differentiation of Treg lymphocytes toward a Th2-like phenotype (23).

Therefore, our aim was to verify the preliminary hypothesis that both IL-33 and IL-13 are higher in patients with diffuse SSc and interstitial lung disease (SSc-ILD) compared both to SSc patients without ILD, as measured through pulmonary function tests (DLco, TLC, FVC), and to healthy controls.

MATERIALS AND METHODS

Patients

This is a single-center cross-sectional study involving SSc patients fulfilling 2013 criteria for SSc proposed by the American College of Rheumatology (24) and classified as diffuse form, according to the classification criteria of Le Roy et al. (25).

Among 90 eligible participants, those having pre-existing respiratory disorders and smokers ($n = 7$), SSc patients with the limited form ($n = 47$) and patients with overlap syndrome ($n = 6$) were excluded. Thus, 30 patients with the diffuse form of SSc were included in the study. Fourteen SSc patients were under treatment with immunosuppressants (11 with mycophenolate, mofetil, 9 with corticosteroids, 3 with azathioprine). Thirty age and sex-matched healthy individuals were included in the study as healthy controls.

Clinical Assessment

Complete demographic and clinical profiles were collected for all participants at enrollement. The modified Rodnan skin score score (mRSS) was measured by summing skin thickness measurements on a scale of 0–3 in 17 body areas (26). The study was approved by the ethical committee of the University of Messina (protocol number 1/2016).

Measurement of Serum IL-33 and IL-13

The serum concentrations of IL-33 and IL-13 levels were quantified using specific enzyme- linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA), according to the manufacturer's indications.

Pulmonary Function Assessment

All participants underwent pulmonary function measurements using a computerized spirometric system (Med Graphics Corporation; St. Paul, Minnesota, USA). Single-breath diffusing capacity for carbon monoxide (DL_{CO}) was also measured and the values corrected for hemoglobin levels.

Radiologic Assessment

Interstitial lung disease was defined as bibasilar interstitial fibrosis on high-resolution computed tomography (HRCT) of the chest. The presence of interstitial lung disease was evaluated by topographically dividing the lung into segments, using the Warrick score which is calculated according to the HRCT extent and appearance. The severity score ranges from normal (0) to all lesion present (15) while the extension score ranges from normal (0) to more than nine pulmonary segments involved (15). The sum of the severity and the extension scores represents the total Warrick score (27).

Statistical Analysis

The Mann–Whitney U test was used to compare IL-33 and IL-13 levels. Fisher's exact probability test was used to compare frequencies, while the bivariate relationships between variables under study were assessed using the Spearman correlation coefficient. Linear regression analysis was used to examine the relationship between two variables. Multiple regression analysis were fitted according to the outcome of interest and analyzed separately due to the limited number of cases. The exploratory approach of the study allowed to analyze IL-13 and Il-33 as independent variables with a single dependent variable each time (DLco, TLC, FVC, HRCT Warrick severity score). A probability (p) value of <0.05 was considered significant. All analyses were conducted using SPSS version 22 (SPSS, Inc., Chicago, IL, USA). Graphs were created using GraphPad Prism 6 (GraphPad Sofware, La Jolla CA, USA).

TABLE 1 | Demographics and outcomes of interest of systemic sclerosis patients and controls.

	dcSSc ($n = 30$)	Controls ($n = 30$)	p
Age, mean ± SD years	58.5 ± 12.4	57.6 ± 13.5	0.78
Women, no. (%)	26 (87)	25 (83)	
Raynaud's phenomenon duration, mean ± SD years	8.8 ± 5	/	
Disease duration (onset of non-RP symptoms), mean ± SD years	5.6 ± 3.8	/	
IL-33 pg/ml, mean ± SD	36.8 ± 23.4	12.4 ± 8.6	<0.0001
IL-13 pg/ml, mean ± SD	0.84 ± 0.65	0.35 ± 0.18	0.0002
Warrick severity score, mean ± SD	4.6 ± 4.31	/	
Warrick extension score, mean ± SD	3.58 ± 3.03	/	
HRCT pulmonary fibrosis, no. (%)	16 (53)	/	
DLCo, mean ± SD	72 ± 19.9	/	
DLCo < 70%, no. (%)	14 (46)	/	
TLC, mean ± SD	75.8 ± 16.5	/	
TLC < 70%, no. (%)	11 (36)	/	
FVC, mean ± SD	78.5 ± 18.3	/	
FVC < 70%„ no. (%)	11 (36)	/	
mRSS, mean ± SD	18.5 ± 6.2	/	
ANA+, no. (%)	30 (100)	/	
Scl70+, no. (%)	12 (40)	/	
ACA+, no. (%)	3 (10)	/	
RNA III+, no. (%)	3 (10)	/	
SRC, no (%)	0 (0)	/	
Costipation, no (%)	3 (10)	/	
Diarrhea, no (%)	2 (6)	/	
Gastritis, no (%)	7 (23)	/	
Proctitis, no (%)	2 (6)	/	
GERD, no (%)	19 (63)	/	

The table shows the demographic features and the outcomes of interest of systemic sclerosis patients with diffuse cutaneous form (dcSSc) and healthy controls. No statistical differences were observed in gender or age between the groups while significantly higher concentrations of both IL-33 and IL-13 were found in dcSSc patients. Additionally, autoantibody profile, disease and Raynaud's phenomenon duration, modified Rodnan skin score (mRSS), pulmonary function tests and Warrick scores of scleroderma patients are reported.
dcSSc, diffuse cutaneous form of systemic sclerosis; RP, Raynaud's phenomenon; HRCT, high resolution computed tomography; DLco, diffusion capacity for carbon monoxide; FVC, forced vital capacity; TLC, total lung capacity; ANA, anti-nuclear antibody; Scl70, anti-Scl70 antibody; ACA, anti-centromere antibody; RNA III, anti-RNA polymerase III antibody; SRC, scleroderma renal crisis; GERD, gastroesophageal reflux disease.

RESULTS

As shown in **Table 1**, 30 dcSSc patients participated in the study (26 women and 4 men; age 58.5 ± 12.4 years) and 30 healthy controls (25 women and 5 men; age 57.6 ± 13.5).

Anti-topoisomerase I (Scl70) antibodies were positive in 12 patients, anticentromere antibodies in 3, anti-RNA polymerase III (RNAIII) antibodies in 3. The median disease duration, calculated from the first non-Raynaud symptom was 5.6 ± 3.8 years.

IL-13 and IL-33 Serum Levels and Disease Outcomes

First, we wanted to assess whether there was any difference in serum levels of IL-33 and IL-13 between SSc patients and controls. We found that IL-13 and IL-33 levels were significantly higher in SSc patients compared to controls. Furthermore, we observed that IL-13 and IL-33 are directly correlated with one another ($r^2 = 0.32$, $p = 0.0009$).

Next, we wanted to assess whether there was any correlation between both interleukins and pulmonary function test and we observed an inverse correlation between each parameter (DLco, FVC, TLC) and both IL-13 and IL-33 (**Figures 1A,B**).

Subsequently, we sought to verify the association between the modified Rodnan skin score and IL-13 and IL-33. In this case, we found a significant direct association only with IL-33 levels (**Figure 1C**), while no significant association was observed for IL-13.

Interleukins and Interstitial Lung Disease: Subgroup Analysis

As a secondary analysis, we divided our study population according to each pulmonary function parameter in higher than 70% predicted and lower than 70% predicted. As shown in **Table 1**, Among the entire study population 16 patients had HRCT signs of interstitial lung disease, 14 had a DLco ≤ 70%, and 11 FVC and TLC ≤ 70%. No differences were observed in age, disease and Raynaud's phenomenon durations, mRSS, GI manifestations, scleroderma renal crisis and autoantibodies

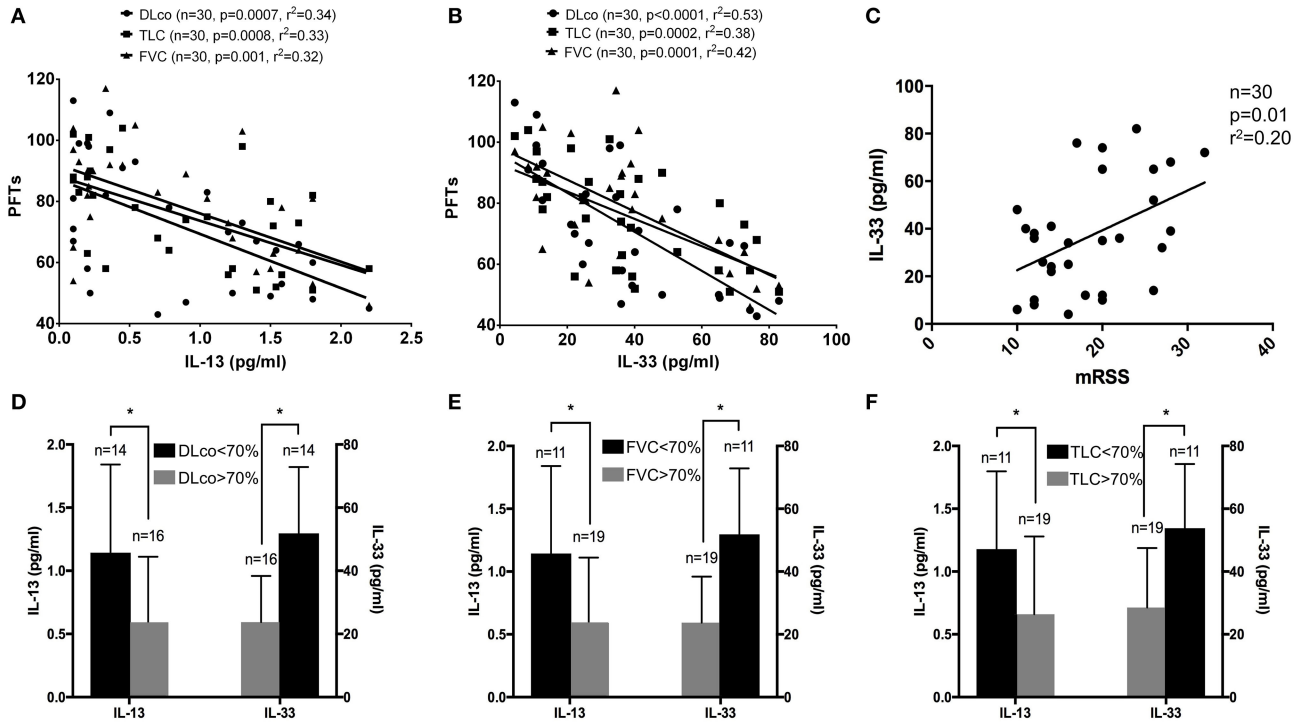

FIGURE 1 | (A–F) Linear regression results for IL-13 and IL-33 and pulmonary function tests: both IL-13 and IL-33 levels were significantly associated with DLco (circle), TLC (square) and FVC (triangle) in systemic sclerosis (SSc) patients **(A,B)**. A significant direct association was observed between IL-33 levels and modified Rodnan skin score in the entire cohort of SSc patients **(C)**. IL-13 and IL-33 were plotted, respectively, on the left and right ordinate of each image to show the difference in concentrations according to the 70% cut-off for DLco **(D)**, FVC **(E)** and TLC **(F)** for SSc patients. *$p < 0.05$.

profile between the groups stratified according to pulmonary function parameters.

Our results showed that both IL-33 and IL-13 are significantly higher in the group of SSc patients with pulmonary function tests lower than 70% (**Figures 1D–F**). Multiple regression analysis, after adjusting for age, showed that IL-13 and IL-33 are associated with DLco (adjusted $r^2 = 0.54$, $p < 0.0001$), with FVC (adjusted $r^2 = 0.37$, $p < 0.001$) and with TLC (adjusted $r^2 = 0.42$, $p < 0.0001$).

In addition, also Warrick severity score was directly associated with both IL-33 ($r^2 = 0.20$, $p = 0.0122$) and IL-13 ($r^2 = 0.16$, $p = 0.032$) when analyzed separately. Multiple regression analysis, after adjusting for age, showed a significant direct association between Warrick severity score and both IL-13 (adjusted $r^2 = 0.21$, $p < 0.014$) and IL-33 (adjusted $r^2 = 0.19$, $p < 0.022$). No relevant association was observed with the Warrick extension score.

DISCUSSION

The usual natural history of SSc-ILD is characterized by a slow decrease in pulmonary function, with a median survival of 5–8 years (28–30). Although some patients experience a rapid pulmonary decline within the first 3 years of disease, in others ILD represents the initial clinical manifestation of SSc. One of the main unmet needs in SSc clinical trial design is the identification of circulating biomarkers that can accurately serve as predictors of interstitial lung disease progression. Our results show that two interleukins, IL-13 and IL-33, closely related to one another, are increased in a specific subset of systemic sclerosis patients with interstitial lung disease, a relevant complication conferring a high risk for mortality and morbidity.

Indeed, a recent report found that IL-13 levels were associated with the severity of restrictive lung disease in SSc patients with early disease (19, 31), while IL-33 induces migration of Th2 lymphocytes and enhances Th2 cytokine production, such as IL-4, IL-5, and IL-13 *in vitro* (6), thus contributing to the production of collagen by fibroblasts (32) and the activation of pro-inflammatory pathways, such as NF-kappaB. In addition, SSc-ILD has several cellular components involved, including endothelial cells, fibrocytes and fibroblasts and immune cells (33).

Apart from the evidence suggesting the relevance of IL-33 in early SSc, we found that the IL-13/IL-33 axis acquires particular relevance as a marker of disease activity of ILD in SSc patients with the diffuse form, with possible implications for IL-13/IL33 as a future treatment target along with its downstream mediator ST2 (6, 12). IL-33 appears to be one of the main factors that increase early during the disease course in SSc (34), mainly induced by activated endothelial cells (22, 35), thus linking its pro-fibrogenic properties to the vascular disarrangement of the disease (14). Indeed, previous studies reported increased levels of IL-33 in SSc and further support its relevance in the diffuse form of the disease and in subjects with ILD, as observed in our study (19).

This study has some limitations: first of all, the number of patients is too low to support definite conclusions although the patient population has specific inclusion criteria. Secondly, since some SSc patients were under immunosuppressive therapy, its influence on interleukins serum levels cannot be determined. Also, the design of the study and the absence of data regarding disease activity does not allow to draw definite conclusions.

In conclusion, our findings support IL-33 role in association with IL-13 in a subset of SSc patients with ILD and with the diffuse cutaneous form and warrant further longitudinal studies to assess the validity of these two interleukins as potential therapeutic targets and biomarkers for severe SSc-ILD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Messina. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GB, AV, SG, SCo, AB, WR, and NI contributed to conception and design of the study. CI, CA, DL, TD'A, AC, SCi, and MN organized the database. GB, AB, NI, DL, and SCi performed the statistical analysis. GB, AV, AB, and WR wrote the first draft of the manuscript. SCo, SG, NI, CI, CA, DR, TD'A, AC, and MN wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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Prospective Studies on the Risk of Rheumatoid Arthritis: The European Risk RA Registry

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Background: The accumulation of risk for the development of rheumatoid arthritis (RA) is regarded as a continuum that may start with interacting environmental and genetic factors, proceed with the initiation of autoimmunity, and result in the formation of autoantibodies such as anti-citrullinated peptide antibodies (ACPA). In parallel, at-risk individuals may be asymptomatic or experience joint pain (arthralgia) that is itself non-specific or clinically suspicious for evolving RA, even in the absence of overt arthritis. Optimal strategies for the management of people at-risk of RA, both for symptom control and to delay or prevent progression to classifiable disease, remain poorly understood.

Methods: To help address this, groups of stakeholders from academia, clinical rheumatology, industry and patient research partners have collaborated to advance understanding, define and study different phases of the at-risk state. In this current report we describe different European initiatives in the field and the successful effort to build a European Registry of at-risk people to facilitate observational and interventional research.

Results: We outline similarities and differences between cohorts of at-risk individuals at institutions spanning several countries, and how to best combine them within the new database. Over the past 2 years, besides building the technical infrastructure, we have agreed on a core set of variables that all partners should strive to collect for harmonization purposes.

Conclusion: We emphasize to address this process from different angles and touch on the biologic, epidemiologic, analytic, and regulatory aspects of collaborative studies within a meta-database of people at-risk of RA.

Keywords: rheumatoid arthritis, prevention, database, multi-center study, observational

INTRODUCTION

The current classification criteria for rheumatoid arthritis (1, 2) published in 2010 make it possible to better define patients at an earlier stage of their disease course in comparison to 1987 ACR criteria. Understanding of how the natural history of RA can be temporally subdivided is no longer limited to early and established RA, but also acknowledges our growing understanding of the pre-disease phases (3–5). Further insights into the development of RA have been formalized into an agreed concept that constitutes the basis for the definition of the criteria of clinically suspect arthralgia (CSA) amongst individuals at risk to develop RA (6). In some individuals certain environmental and/or genetic risk factors can lead to the awakening of systemic autoimmunity, the formation of autoantibodies and musculoskeletal (MSK) symptoms, before the onset of arthritis (7–11). In the next step, patients that eventually develop clinically apparent arthritis might then be classified at some point as having RA. Based on this concept (**Figure 1**), it is of importance for further understanding of the gradual emergence of RA and for the development of preventive strategies for arthritis that cohorts of individuals with musculoskeletal symptoms and RA-associated immunity are created. When not considering the ACR/EULAR classification criteria for RA (12), the decision when an individual "at-risk" has formally become a patient with arthritis can be shifted along this above explained continuum. The identification or naming of arthritis is also depending on assessment measures used in the work-up of these individuals at-risk, since arthritis might be identified using additional (imaging) techniques at early points (13).

The Rheuma Tolerance for Cure (RTCure) consortium started in September 2017 aiming to change the treatment paradigm from late and non-specific to early and specific (**Figure 1**) and to improve understanding of the at-risk phases of RA and factors determining the transition toward disease. One major task for the consortium is to develop and populate a longitudinal register with defined inclusion criteria, methods for surveillance of included individuals and defined outcomes (arthritis). This collaborative and harmonized "pool of at-risk individuals" could be used as a platform for recruitment into trials testing preventive therapies and strategies. The overall aim of the RTCure project is to contribute to the development of therapies that affect the adaptive immune system via tolerization and other strategies, and to enable such therapies to be tested and implemented in very early phases of the disease, preferably even before joint inflammation has occurred. This endeavor is built on the premise that MSK complaints (i.e., stiffness, numbness, reduced function) are key symptoms that cause a person to seek medical advice, and therefore represent a feasible starting point for considering study enrolment. However, the terms "arthralgia" and "joint pain" can be used synonymously (we will use the term "arthralgia") and inclusion in the register must therefore also be built on other criteria, which in the present setting is frequently the presence of autoantibodies to citrullinated proteins/peptides (ACPA) and/or rheumatoid factor (RF), but can also be a set of clinical and imaging determinants as described for the term "arthralgia

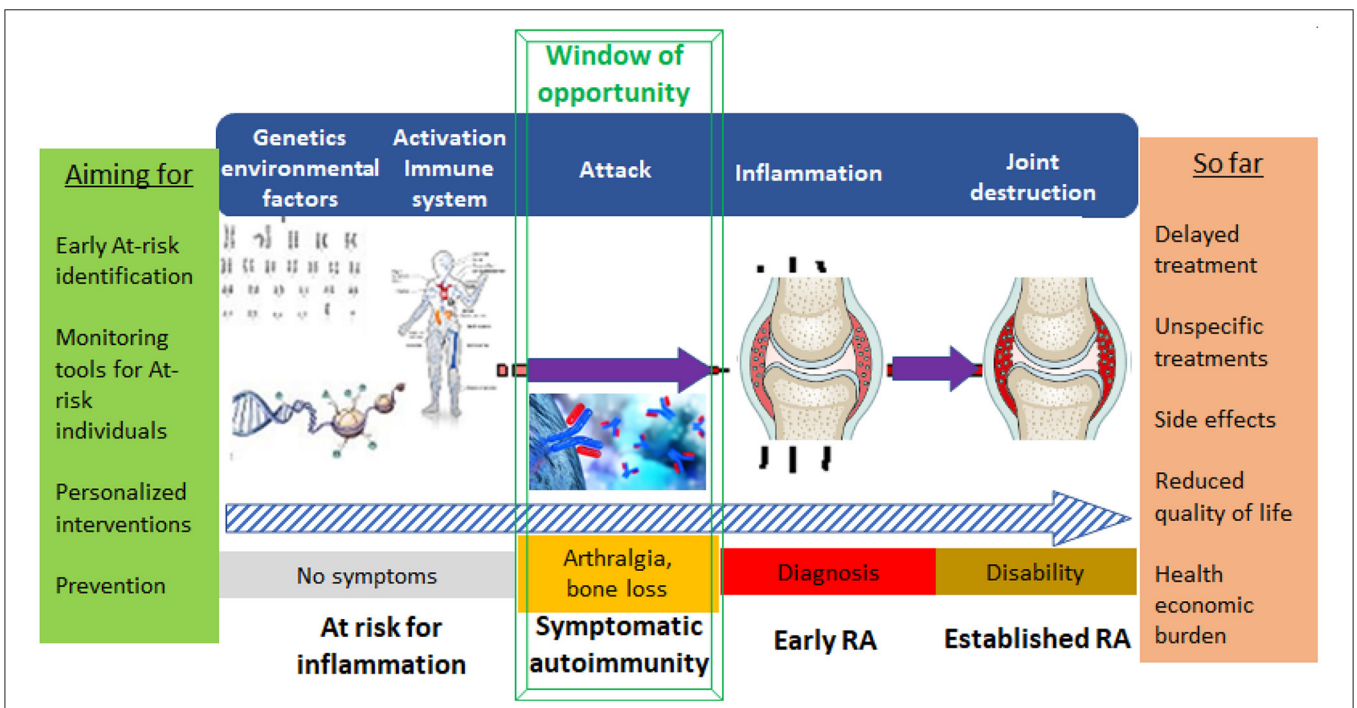

FIGURE 1 | Visualizing the development of RA and key aims in future healthcare management of people in different stages/phenotypes at-risk of developing arthritis/RA.

suspicious for the progression to rheumatoid arthritis." Other rheumatic and non-rheumatic causes should be ruled out before combining arthralgia with other factors to better characterize the at-risk state (i.e., morning stiffness, family history of RA, involvement of the metacarpophalangeal joints) (6). The risk of developing RA in such settings is being studied prospectively by several different European national and local initiatives.

In the current report, we outline consensus work to derive core outcomes of interest for a European registry of individuals at risk of RA, differences and parallels between those European initiatives it encompasses, and the challenges of building and managing such a registry.

METHODS

Developing a Core Data Set to Collect in At-Risk Individuals

The RTCure Research Consortium, created in 2017 with funding from the IMI ("Innovative Medicines Initiative 2 Joint Undertaking") assembled 20 academic and industry stakeholders as well as at risk individuals and patients with RA (www.RTCure.com) with the aim of earlier detection and prevention of RA. To help synchronize future work one major task was to define the populations with a risk to develop RA and to agree on a minimum core set of variables that should be collected and reported in a future mutual registry. During working group meetings from 2018 to 2020, different definitions were discussed and reassessed regarding feasibility with the RTCure partners interested in contributing to this common registry and with the aim to also include other partners once the registry has been established. The final set of variables was agreed upon together with the small-medium enterprise (SME) Zitelab, experienced in creating registries. Zitelab was then tasked with managing the infrastructure of the database.

Set-Up of a Multi-Center Registry

One major emphasis in RTCure was to establish an infrastructure that will permit coordinated clinical trials of immunotherapy in seropositive individuals at-risk for RA, but yet without signs of joint inflammation. In a collaboration between several academic partners and the SME ZiteLab, an electronic registry has been developed that will allow consolidation of registries of individuals at-risk of developing RA across multiple academic partners. The registry platform is also a tool to help harmonize and balance the different reporting of collected variables. The contribution of ZiteLab is to provide an IT-based research infrastructure as an integral research partner of the collaborations (14, 15). All participating centers had received local ethical approval for their registry projects and additional documents by their respective legal departments for data sharing have been developed.

Existing Cohorts and Recently Established Programs

We describe and compare cohorts of five RTCure partners (Karolinska Institutet, Medical University of Vienna, University Clinic Erlangen, Leiden University Medical Center and University of Newcastle). Established at different points in time, they represent overlapping groups amongst the heterogenous population of interest to RT-Cure. Communalities and differences are reported descriptively.

TABLE 1 | Agreed minimum core set to report in individuals at-risk studies.

Baseline/demographic characteristic parameters		
Sex		Class of pain medication
Year of birth		Available biologic specimen
First rheumatic consultation		Height
Inclusion year		Weight
Symptom onset		Smoking
Type of patient consent		Alcohol
Follow-up variables		
Any treatment		
Laboratory Markers		
	C-reactive protein	
	Erythrocyte sedimentation rate	
	Anti-CCP	
	IgM rheumatoid factor	
Objective markers of disease activity		
	Swollen joint count 66	
	Tender joint count 68	
	Disease activity score	
	CDAI	
Patient-reported outcomes		
	Pain	*
	Fatigue	
	Patient global assessment	
	Health assessment questionnaire	
	Morning stiffness	
	Day/time of most severe symptoms	
Evaluator global assessment		

RESULTS

Derived Core Outcomes for People At-Risk

Since 2018 members of the RTCure consortium have met on several occasions, together with the ZiteLab managers, and defined what types of data are recommended to be included in the registry. This was an iterative process, identifying commonalities between the existing site-specific registries, and further defining what data should be included in future prospective studies.

During discussion rounds at working group meetings, at conferences and via email, the core set of variables of highest interest was developed and finally agreed during the working group meeting at EULAR 2019 (**Table 1**). The pre-work of the EULAR TF on defining CSA was considered in the discussions concerning the construction of this set (6, 16). Including information on autoantibodies and eventual fulfillment, or the degree of fulfillment, of CSA criteria is of importance, since they are among the most promising target populations for preventing RA (4, 17, 18).

One Database for Different At-Risk Cohorts

The efforts toward data harmonization have led to the construction of a functional registry and web-based interface. The technical backbone of the RTCure at-risk-registry was built between 2018 and 2019. During a 2-day consensus event, with participation from each of the involved centers, an extended list of all candidate-variables was described with a reference to its use in either EULAR recommendations, prior decisions of the consortium or existing (or planned) data collections in the participating centers. In a consensus-process, each variable was given an importance-score between level 1 and 4 (4 being the lowest). Variables that were assigned an importance-score of one eventually were then termed as the core set variables. During the consensus event, an information technology (IT) system was built resulting in a web-based system where each of the centers—having secure access to their own part of the system—can see the decisions expressed within metadata sheets of variables, upload facilities with validation of uploads, web entry forms and search facilities (19). In the development phase all variables suggested were included in a first version. Then based on further dialogue every variable was tagged with an importance score.

Growing with the needs of the collaborators, the structure was continuously fine-tuned with improved access to a metadata-sheets explaining details of the data-model, hence the variables, types and parameter values. Furthermore tools for dialogue-oriented clarifications of potential differences between the agreed data-model built in the system and the available local data of each partner has been put in place. Since the database tool and the dialogue and the metadata sheet validating the upload are based on the same excel sheet (automatically parsed into different use-cases) the process was highly efficient. By using the approach of an "importance scores" still the database contains a part where all participating partners agree and other parts where variables are not used or shared by all partners. In early 2022 a tool for accessing summary data across the different cohorts is being added.

This early and instant availability of a functional IT-system was based on reuse of components from a pre-existing IT-system used by the global myositis community (14) and EuroSpA collaboration (15).

During the following months the main barrier to overcome related to establishing GDPR-compliant risk assessments which conformed with the different traditions between the various participating University Hospitals, and Data Processor Agreements (DPA) between the University Hospitals and ZiteLab as a pre-condition for data uploads.

An important challenge, even within a consortium, was overcoming ethical and legal requirements to be able to upload data into the registry. Ethical issues are different in every country due to country-specific procedures and because of different underlying data-collections from which the data-extractions are done. Legal issues relate to (i) different procedures in risk-analysis, (ii) different classification related if data processing agreements are needed, (iii) capacity problems in the legal units and (iv) unclarified internal data transfers agreements between university units and hospital units. The legal implementation strategy involved first establishing a completed risk assessment and DPA-agreement with one of the University Hospitals and then rolling out the same set of documents to the remaining centers, with slight adaption to local regulations and governance. As example in Sweden clarification of the related role of University Hospital and the Research Institute, In Germany the relation to Secrecy Obligation according to § 203 German Criminal Code (StGB). In the United Kingdom the Data Protection Impact Assessment had to use an extended template.

During a period of 2 years these negotiations led to contracts with the cohort partners and uploads of datasets from two partners, with expected completion of the full process of first data uploads in the first quarter of 2022.

Patient and Public Involvement

Patient Research Partners (PRPs) are defined as "persons with a relevant disease who operate as active research team members on an equal basis with professional researchers, adding the benefit of their experiential knowledge to any phase of the project" (20). Two PRPs with established RA from the UK were involved with the set-up of this registry and attended registry-specific discussions during the EULAR annual congresses and the RTCure annual meetings from 2018 onwards. At the EULAR 2018 meeting, one PRP questioned why seronegative people were not going to be included strategically in the registry database. Following a discussion between all stakeholders, it was decided that although the emphasis as prospective entry criterion is set on seropositivity, all data in existing registries would be included to the RTCure at-risk-registry independent of detected autoantibodies.

European Cohorts: Mutual Aims and Differences

Karolinska Institutet

At the Karolinska Institutet and the Karolinska University Hospital, the Risk RA prospective research program to study individuals with MSK symptoms and systemic autoimmunity, specifically ACPA has been established in year 2015. It aims to understand how symptoms and biomarkers evolve over time in individuals who develop arthritis within 3 years compared with those who do not. Particular emphasis lies on the development of predictors for ACPA-positive arthritis development and on the presence of pain and fatigue over time in individuals in the cohort. The program includes assessing the impact of genetics and environmental and lifestyle factors on the risk for arthritis development and on the symptomatology during the observation time, using an extensive questionnaire for lifestyle and environment and genome-wide association studies (GWAS)-based genetic analysis. The RISK RA cohort encompasses about 300 people, who are followed up through the RISK RA register over 3 years in a predefined schedule or until the onset of arthritis (**Table 3**). The individuals are identified in primary care as individuals with MSK complaints, suspicious for a rheumatic disease and referred to the rheumatology clinic. At the clinic, clinical and ultrasound (US) examinations are performed to evaluate joint inflammation. Tendon and bone involvement is additionally checked by means of US but not taken into account for deciding whether an individual would

TABLE 2 | Criteria necessary for inclusion into the individual at-risk cohort programs.

Criteria	KI	MUV	UKER	LUMC	UNEW
CCP positivity	+	±	+	±	+
RF positivity	±	±	±	±	±
CCP AND/OR RF positivity	±	+	±	±	±
EULAR CSA criteria fulfilled	±	±	±	±	±
Clinical suspicion by a rheumatologist*	+	+	+	+	±
No glucocorticoids reveived	+	+	+	+	+
SJC 66 = 0	+	+	+	+	+
No clinical arthritis	+	+	+	+	+
No synovitis detected by using US	+	±	±	na	±
Presence of tenosynovitis in US	±	±	±	na	±
Structural changes in US	±	±	±	na	±

+, criteria must be fulfilled, ± is allowed to be fulfilled; na, not assessed.
*CSA, Clinical suspect arthralgia for progression to RA; *Meaning that an experienced rheumatologist concludes based on the assessment that this patient is at risk to progress toward the development of rheumatoid arthritis; KI, Karolinska Institute; MUV, Medical University of Vienna, in regard to the ASPRA cohort; UKER, University Clinic Erlangen; LUMC, Leiden University Medical Centre; UNEW, Newcastle University.*

be included or not. If any signs of joint inflammation (arthritis on either clinical or US investigation) are identified, the person is diagnosed with arthritis in need of immediate treatment and is followed-up according to existing national and international guidelines. To be able to study the risk of arthritis onset, strict inclusion criteria (**Table 2**) are applied for the program making only individuals with minimal US changes able to participate. Hence, individuals scoring > 1 by gray scale and/or ≥ 1 by power Doppler using the EULAR-OMERACT scoring system (21) are excluded from participation. Individuals that do not have signs of joint inflammation are then invited to participate in the RISK RA program. Neither before inclusion or during the follow-up individuals are allowed to receive glucocorticoids (GC) or DMARDs. During the program individuals are advised on symptomatic pharmacological (NSAIDs) and non-pharmacological treatments (e.g., physiotherapy). The follow-up strategy includes both on demand rapid visits if symptoms worsen and routine follow-ups with at least yearly visits. If clinical or US arthritis develop during the follow-up period, people are treated according to the national guidelines for RA treatment.

Medical University of Vienna

PRERA

The PRERA cohort is a closed cohort without further recruitment or follow-up. This study was conducted between 2010 and 2018. Included individuals were followed-up over 5 years. None of them developed classifiable inflammatory arthritis. People were enrolled *via* the Austrian free annual health examination, independent of the presence or absence of MSK symptoms, but excluding those with established inflammatory rheumatic conditions. Seropositive individuals were matched for sex and age with seronegative individuals and have undergone assessment of RF, ACPA, RA-33, lifestyle and family history at baseline and routine clinical and laboratory assessments every 6 months. **Table 3** reports on data of 98 individuals that accepted the invitation to participate. Of note, the rates of progressors reported in **Table 3** relate to swollen joints due to any reason and should not be interpreted as progression toward RA. However, since only 45 individuals continued within this program after 2 years, it is to be expected that in the healthy asymptomatic drop-out population, no rheumatic condition so far manifested.

ASPRA

The Vienna Arthralgia Suspicious for Progression to Rheumatoid Arthritis (ASPRA) registry was started in August 2020 within a specialized outpatient program of the MUV, including seropositive individuals with arthralgia, without clinical arthritis in a structured follow-up management program. The ASPRA program is already based on the agreed core data for the meta-database originated in the RTCure project and data on radiologic changes (assessed by US and micro computed tomography—micro-CT), as well as lung function and cardiovascular risk factors which are longitudinally collected in addition. Inclusion in the program is based on the presence of positive CCP or RF tests in individuals with MSK complaints, without clinical arthritis, but the suspicion of the rheumatologist for the risk of progressing to RA (**Table 2**). At-risk individuals are invited to remain in the program over 5 years with visits twice yearly or until the onset of any classifiable rheumatic condition. All study participants complete questions on CSA (6), the SPARRA questionnaire (22) and take part in the biobanking program of the division for retrospective analyses of molecular targets of interest. GCs or DMARDs cannot be received before inclusion or during the at-risk phase. Similarly to the program at KI symptomatic therapy is offered. In comparison to the historical PRERA study that invited seropositive and control individuals without the need of symptoms recruited through referrals from yearly health check-up offered by the public health system, this ASPRA registry has a higher potential to identify individuals who go on to develop arthritis. Around 1 year after start of this program 4 out of 28 patients have developed classifiable RA (**Table 3**).

University Clinic Erlangen

To explore the development of arthritis, a RA at-risk cohort (IRACE cohort Individuals at Risk for Arthritis Cohort Erlangen) was initiated in 2011. This prospective cohort includes people with serological evidence of CCP antibodies with or without MSK symptoms (**Table 2**). Two participants are without MSK symptoms but only have rheumatoid nodules. Individuals with clinically apparent arthritis (at least one swollen joint with synovitis in clinical assessment in the 66 joint count, performed by an experienced rheumatologist) are excluded. Currently, the RA-at-risk cohort consists of 175 at-risk individuals (as of November 2021). **Table 3** provides an overview on participant characteristics with follow-up time longer than a year. Individuals with permanent GC therapy or GC therapy at the time of initial presentation are not included into the cohort. Individuals within the program should not require GC at the time point of a visit. However, intake of GCs in between visits for no more than 2 days is permitted.

TABLE 3 | Overview of risk cohorts included in the RTCure at-risk registry infrastructure, with follow-up data in July 2021.

		KI	MUV—PRERA	MUV—ASPRA	UKER	LUMC	LUMC (ACPA+)	UNEW
Number		268	98	28	106	645	91	32
Age	median (iqr)	48 (36–58)	57 (47–64)	53 (40–57)	50 (40–58)	44 (34–54)	52 (39–57)	50.5 (34–57)
Female sex	*n* (%)	212 (79)	55 (61)	22 (78)	73 (68.9)	490 (76)	72 (79)	22 (69%)
Symptom duration (months)	median (iqr)	22 (10–50)	missing	14 (6–12)	37.5 (26.8–96)	4 (2–9)	5 (3-12)	4 (2–9)
Ever smoked	*n* (%)	150 (58)	51 (56.7)	10 (59)	59 (55.7)	326 (58)	56 (71)	16 (50%)
Never smoked	*n* (%)	110 (42)	36 (36.7)	7 (41)	42 (39.6)	237 (42)	23 (29)	3 (9%)
Current smoker	*n* (%)	44 (17)	24 (24.0)	6 (35)	35 (33.0)	120 (21)	24 (30)	9 28%)
Previous smoker	*n* (%)	106 (41)	29 (30.0)	4 (24)	24 (22.6)	206 (37)	32 (41)	7 (22%)
Pain (VAS, 0–100)	median (iqr)	26 (10–52)	0 (0-9.5)	5 (2.5–7)	17 (3–33)	5 (3–7)	4 (2-6)	40 (0–70)
Patient Global Assessment (VAS, 0–100)	median (iqr)	28 (6–51)	0 (0–5)	3 (2–7)	12 (1–33)	3 (2–5)	3 (1–6)	37 (20–70)
Evaluator Global Assessment (VAS, 0–100)	median (iqr)	0 (0–1)	0 (0–0)	1 (0–2)	3 (2-12)	missing	missing	10.5 (1-57.5)
Morning stiffness ≥ 60 min	*n* (%)	57(28)	0 (0%)	3 (13)	9 (8.5)	212 (35)	30 (35)	9 (28)
SJC28	median (iqr)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
TJC28	median (iqr)	0 (0–2)	0 (0–0)	2 (0–5)	0 (0–2)	3 (1–6)	2 (0–3)	1 (0–3)
CRP (mg/dl)	median (iqr)	0.1 (0.1–0.4)	0.15 (0.07–0.27)	0.145 (0.12–0.26)	0.52 (0.39–0.55)	0.30 (0.30–0.47)	0.36 (0.30–0.72)	0.4 (0.4–0.6)
ESR (mm/h)	median (iqr)	11 (5–19)	10 (6–15)	11 (7–20)	11.5 (7–17)	6 (2–14)	11 (6–24)	8.5 (5–15.25)
Frequency anti-CCP positivity	*n* (%)	268 (100)	3 (3.3)	6 (37.5)	84 (79)	91 (14)	91 (100)	32 (100)
Anti-CCP titre (times relative cut-off)	median (iqr)	10 (3–100)	0.8 (0.4–1.9)	1.8 (0.7–136)	88.8 (15.8–1574.4)	0.14 (0.10–0.14)	29 (7–49)	233.5 (20.5–301)
Frequency RF positivity	*n* (%)	33% (88)	27 (30)	14 (87.5)	60 (56.6)	135 (21)	70 (77)	21 (65)
RF titre (times relative cut-off)	median (iqr)	0 (0–1.6)	0 (0–11.6)	35 (18–48)	22 (11.6–62.8)	0.23 (0.11–0.66)	5 (1–18)	61 (38–130.5)
Follow-up time (months)	median (iqr)	19 (12–26)	24.5 (6.8–55.0)	4 (2–6)	2 (1–5)	24 (11–26)	4 (1–23)	44 (28–82)
Arthritis progressors 0–6 months	*n* (%)	26 (10)	7 (9)	4 (14)	22 (21)	73 (13)	36 (50)	7 (22)
Arthritis progressors 0–12 months	*n* (%)	41 (17)	8 (12)		27 (26)	77 (14)	37 (51)	9 (35)
Arthritis progressors 0–24 months	*n* (%)	67 (44)	10 (22)		34 (36)	88 (17)	41 (59)	13 (52)
Ever arthritis progressors	*n* (%)	75 (28)	10 (10)	4 (14)	41 (38)	98 (15)	44 (48)	17 (53)

KI, Karolinska Institutet; MUV, Medical University of Vienna; UKER, University Clinic Erlangen; LUMC, Leiden University Medical Centre; UNEW, Newcastle University; VAS, Visual Analogue Scale; TJC, Tender Joint Count; SJC, Swollen Joint Count. (Progression rates of PRERA relate to swollen joints due to any reason and not to progression to RA).

Participants are seen between every 3, 6, or 12 months during their clinical routine appointment. After informed consent, basic characteristics such as body mass index, periodontitis, family history of RA, medication, comorbidities, alcohol intake, smoking as well as 66/68 swollen/tender joint count are obtained. Visual analogue scales (VAS; scores of participants and physicians), DAS28 and Health Assessment Questionnaire (HAQ) are available. For basic research purposes, serum and full blood samples are collected and stored. Endpoints are: ACR-EULAR Classification criteria/clinical apparent arthritis. Timespan between the first symptom that led to a person seeking healthcare to diagnosis is calculated. Within the at-risk program, individuals should further be recruited to interventional trials. All receive symptomatic (NSAIDs) therapy as required, as well as non-pharmacological treatment intervention, like education on life-style and diet.

The strength of this prospective observational cohort is the systematic linkage of clinical data with serological biomarkers and state-of-the-art MSK imaging, such as MSK US, HR-pQCT (high resolution peripheral quantitative computed tomography) 1.5 Tesla, high-field 7 Tesla magnetic resonance (MRI) or innovative metabolic imaging approaches such as Fibroblast activation positron emission tomography (FAPI-PET) as in current research projects (13, 23–30). This cohort serves as a source for prospective interventional trials such as the ARIAA trial (EUDRA-CT 2014-000555-93) to study abatacept in the context of preventing or delaying disease onset in RA-at risk individuals with subclinical signs of inflammation as judged by MRI (31). The primary endpoint of this trial, which involves a 6-months treatment phase with abatacept or placebo as well as 12 months follow-up, is defined as an improvement in at least one of the assessed MRI inflammation parameters. Additional questions that are addressed include the progression to clinically overt arthritis of these at-risk individuals upon abatacept treatment and during the follow up period.

Leiden University Medical Centre

The Leiden Clinically Suspect Arthralgia cohort includes consecutive patients presenting with CSA. People with arthralgia of the small joints for <1 year that is considered suspicious for

progression to RA are included at their first visit to the outpatient clinic, thus before any blood tests have been performed (**Table 3**). People are not included if the rheumatologist considered another explanation for their arthralgia (e.g., osteoarthritis or fibromyalgia) more likely than imminent RA. Presence of clinical arthritis (joint swelling) also precludes CSA by definition. In line with national guidelines for general practitioners (GPs), GPs are discouraged to perform ACPA-testing themselves but are encouraged to refer patients in case of any suspicion of imminent RA. Hence, inclusion is mostly done without knowledge of the results of additional investigations (**Table 2**). Treatment with GCs is not allowed before entering the program or during follow-up. Follow-up visits are performed at 4, 12, and 24 months and more regularly in case of increased symptoms. During follow-up, CSA-patients are not treated with DMARDs or GCs but symptomatic treatment with NSAIDs or analgesics is possible. At each study visit patient-reported outcome questionnaires are completed, physical joint examination performed and blood samples taken. In addition, an MRI of small joints is conducted at baseline. Patients are followed for development of clinical arthritis, confirmed with joint swelling at physical examination by the rheumatologist. Fulfillment of classification criteria is noted. This strategy allows to include both autoantibody positive and autoantibody negative RA in the pre-arthritis stage of RA (32).

Newcastle University

The Newcastle "At-Risk of RA" cohort is a defined sub-cohort within the Northeast Early Arthritis Cohort (NEAC)—itself an unselected, observational inception cohort of consecutive, consenting individuals referred from primary care to the Suspected Inflammatory Arthritis service at Newcastle Hospitals NHS Foundation Trust (**Table 3**). Primary care physicians are encouraged to refer such individuals without the need to undertake blood tests (including autoantibodies), since these are routinely performed during secondary care assessment. All such individuals receive two initial assessment appointments 1 week apart. At the first visit, recording of detailed baseline demographic and clinical parameters is undertaken, along with MSK US assessment. At the subsequent visit, people are reviewed by a consultant rheumatologist with access to all results, and assigned an initial clinical diagnosis from a dropdown menu of possibilities that includes "ACPA+ arthralgia." Individuals placed in this category by their consulting rheumatologist (confirmed to have 0 recorded swollen joints out of a total of 74 assessed and a positive anti-CCP2 test result according to routine laboratory testing are defined eligible for inclusion in the "At-Risk of RA" NEAC sub-cohort (**Table 2**). Enrolled individuals are subject to routine care with clinic visit frequency and treatment is at the discretion of their consulting rheumatologist. Treatment with GCs, as well as DMARDs prior to enrolment are not allowed and during follow-up until development of clinical arthritis GCs are discouraged and DMARDs not prescribed. Follow-up data including tender/swollen joint counts and VAS are recorded, data being routinely captured through the electronic patient record (EPR) which is linked to a bespoke database at Newcastle University for research purposes. There is an opportunity for biological sampling for research at baseline and follow-up (33). The development of arthritis represents the end-point (defined as joint swelling confirmed by a rheumatologist); fulfillment/non-fulfillment of RA classification criteria is also recorded for purposes of the current registry, and any immunomodulatory therapeutic interventions are also logged. The overall strategy ensures that prospective data reflect routine, consultant-led care at a single center (34).

Synthesis of Cohorts

These cohorts represent different stages of the longitudinal development of RA with broad clinical characterization and sample collection, allowing in-depth studies on the immune events responsible for each of these stages (**Figure 2**). Data on environmental exposures (specifically smoking) and genetic predisposition (genotyping) as well as biological samples (including blood cells, serum, DNA and RNA preparations) are available for most of the individuals in the cohorts. In selected individuals, we also have access to tissue samples including mucosal samples. The described data is already available in some of the cohorts (such as antibody testing and genotyping in the Karolinska RISK RA cohort and the Leiden early arthritis clinic). This heterogeneity at inclusion as well as the individual cohort endpoints can be overcome by harmonized data reporting. For example, the individuals with clinical arthritis in the at-risk cohort at KI can be pooled with those of other cohorts, since all cohorts contain data on the occurrence of clinical arthritis. When we apply the same timeframe of follow-up results, we can see that the arthritis progression rates across the cohorts become increasingly similar with the seropositive individuals showing progression rates between 44% and 59% within 2 years of follow-up. Following the dogma of harmonized reporting, individuals across all cohorts can be divided into subgroups either fulfilling the criteria of CSA, or overlap of presence for predefined autoantibodies or imaging features at time of inclusion. By collecting this within the RTCure at-risk registry, information regarding samples sizes of different sub-groups can be checked easily, quickly and conveniently.

DISCUSSION

Today, no therapies are approved specifically for the treatment of clinical syndromes that precede RA in individuals with early evidence of autoimmunity, or for the prevention of RA. An important objective in studying at-risk individuals is to enable accurate identification of those with certain types of immune reactions and other biomarkers associated with clinical symptoms that together suggest a high-risk state for progression to clinically evident RA. One major aim of the described registry was to create an infrastructure, *via* a European collaborative network, that can catalog cohorts of such symptomatic patients at high risk of RA who could be enrolled into clinical trials aimed at preventing progression to RA. A specific goal is to create opportunities to selectively target therapies against disease-inducing immune reactions. The program also enables a more rapid diagnosis of arthritis in those individuals included in the register who have regular contact with rheumatologists. Here,

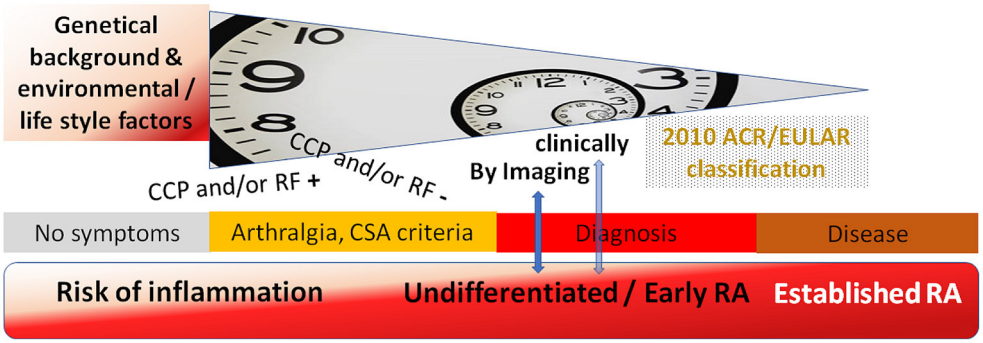

FIGURE 2 | Overview of study time points in the at-risk programs, following up antibody (CCP and/or RF) positive and negative individuals until occurrence of arthritis detected by imaging (KI) or clinically (MUV, UKER, LUMC, UNEW), potentially with overlap to classification as RA according to the 2010 ACR/EULAR criteria.

BOX 1 | Research agenda.

- Better understanding of the trajectory to RA, in dependency of different phases or substages
  - Understanding of the role of risk factors in symptomatic individuals without signs of inflammation in imaging and with signs of inflammation in imaging
  - Differences in risk factors between ACPA positive and negative individuals, and evaluation of a common path
- Developing of a validated risk stratification method to be used in clinical practice to support future trials and to support communication with regulatory agencies
- Defining the different outcomes and their interrelationships: imaging arthritis, clinical arthritis, clinical arthritis that remits spontaneously, persistent clinical arthritis
- Defining relevance of changes in regularly assessed outcomes in at-risk programs
- Contributing to clinical trials aimed at prevention of arthritis and alleviation of symptoms in the at-risk phase
- Defining at-risk Individuals view of acceptable risk/risk period to consider preventative treatment in the development pathway of RA

it will be possible to eliminate most currently encountered patient-related and doctor-related delays in the diagnosis and treatment of arthritis, enabling intervention for undifferentiated inflammatory arthritis very early, with the aim to prevent progression to the disease classified as RA.

In order to further develop this concept from a research-based registry to routine care, it is paramount to work together between several different stakeholders with patients with RA and individuals at-risk for developing RA. This requires close collaboration between academia, care providers and industry: all these networks exist within the RTCure consortium and will feed into the registry's outputs including clinical trials and, ultimately, implementation of guidelines for prevention of RA in widespread clinical practice.

A fundamental laboratory finding that provides a scientific basis for the RTCure program and the registry, is that development of RA-specific autoantibodies against proteins/peptides post-translationally modified by citrullination (ACPA) or other modifications (collectively named AMPAs) precede the onset of joint inflammation by many years. An increase in titers, epitope spreading and autoantibody isotype switching occurs before onset of joint inflammation; conversely, very few patients "seroconvert," i.e., develop autoantibodies, after onset of disease (35–37). The presence of ACPAs also coincides in most cases with the presence of rheumatoid factors (RF) at diagnosis and the disease subset positive for either ACPA or RF, or both, is nowadays conventionally labeled as "seropositive RA." Notably, in the risk phase there seem to be a higher proportion of ACPA positive individuals that are RF negative. Once at diagnosis relatively few patients seroconvert from positive to negative during treatment (38). These and other observations have underpinned concepts of autoimmunity in the aetiopathogenesis of RA, implying causality. Autoantibodies like ACPA are components of immune complexes capable of activating immune effector cells (e.g., osteoclasts) to trigger pathological reactions (bone loss) which in turn leads to clinical symptoms (13, 39, 40). Furthermore, seronegative patients are in many cases not truly seronegative. ACPA fine-specificities, IgG/IgA RF or anti-carbamylated, as well as other AMPAs can be found in some individuals negative for routine anti-CCP and/or RF tests. It seems that the HLA-DRB1 SE is associated with the formation of ACPAs, whereas smoking has its major role in individuals positive for both RF and ACPA, some indications highlight smoking with the occurrence of RF in seronegative disease (41, 42).

All the described programs that are summarized within the RTCure at-risk registry have set different emphasis on different research questions. This can be seen as advantageous for exploring the pathway of at-risk individuals holistically. One crucial point in these programs is the embedment into structures of the healthcare systems of the respective countries/regions to represent the local standard of clinical care that might help in having at-risk individuals feel more comfortable in this limbus of maybe developing or not developing the disease (5). Every cohort-specific approach for setting inclusion and exclusion criteria has advantages, when taking local circumstances into account. The selection of only at-risk individuals with CCP

positivity homogenize individuals under observation (genetic and environmental exposure), leading to higher observed progression rates, but is not representative for all patients with RA that are currently treated in usual care (43). Setting less stringent inclusion criteria (e.g., not mandating seropositivity and/or the detection of subclinical inflammation) allows answering questions of common rheumatoid arthritis symptoms (both seropositive and seronegative variant) and assessments (44). However, from a helicopter perspective we can better grasp that our current problem is to investigate many factors taken together for derivation of better prediction estimates. This poses a challenge for individual cohorts and can only be addressed by studying combined data in a concerted effort.

The cohorts and the European RTCure at-risk RA Registry will allow identification of candidates for clinical trials by screening within the core data set assessments that the partners have agreed on. Such studies are already ongoing (the Treat Earlier study using methotrexate in people with CSA with subclinical joint inflammation in the Netherlands, the APRIPPA study using abatacept in people with seropositive arthralgia in the UK, the ARIAA study using abatacept to treat seropositive arthralgia in Germany and the PREVENT RA study using bisphosphonates to treat pain in seropositive individuals with MSK complaints in Sweden). Currently two thirds of patients fulfilling RA disease classification criteria are characterized by the presence of autoantibodies that have been post-translationally modified. As more disease-relevant post-translational modifications are discovered, the proportion of seropositive RA patients will likely increase (42). This means that in the future more autoantibody-positive at-risk individuals could be eligible for intervention studies. Detecting these autoantibodies early in the disease course has clinical value in at-risk cohorts since they identify individuals at highest risk of developing RA (45).

Only recently, the European Alliance of Rheumatology (EULAR) has published points to consider for conducting clinical trials and observational studies in at-risk individuals (46). The different outcomes for assessment agreed on within our consortium are also named in this recommendation paper, which also reflects the different phases in the development of arthritis, and the foci that are set in the contributing individual registries. As in every collaborative registry also the RTCure at-risk RA Registry is not fully populated with all entries of variables available but has agreed on a core set to work with considering also availability and feasibility. Relevant Items, like ethnicity and family history may also be reported in this registry, but at this point have not been deemed sufficiently available and of interest to the overlap of all processed items among the individual cohorts. Our registry remains dynamic and welcomes further collaboration and inclusion, which over time logically leads to adaptations of the core set.

Taken together, the register for at-risk for RA individuals and the harmonization of clinical data, biobanking and "omics" data for risk estimation, patient stratification and disease monitoring, will provide an internationally unique resource for understanding the longitudinal development of RA (**Box 1**), and also provide the pharmaceutical industry and academia with a potential to conduct clinical trials intended to prevent the development of RA.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the Medical University of Vienna, Ethical Committee of Stockholm, Ethik-Kommission der Friedrich-Alexander-Universität Erlangen-Nürnberg, Medical Ethical Committee of the LUMC and Newcastle and North Tyneside 2 Research Ethics Committee.

AUTHOR CONTRIBUTIONS

PS, AH, AK, AH-vM, AP, DS, GK, MJ, NK, LK, and AC were involved in data collection, handling, and interpretation. The manuscript was drafted by PS and complemented by input from all authors. All authors contributed to the set-up of this project. The final version was approved by all authors.

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Insights Into the Concept of Rheumatoid Arthritis Flare

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Identification of a pathological change in the course of systemic chronic immune-inflammatory diseases is key to delivering effective treatment strategies. In this context, one of the most compelling issues is the concept of flare. The multifaceted expression of disease activity in rheumatoid arthritis (RA) makes it challenging to provide an omni-comprehensive definition of flare, encompassing the pathology's different objective and subjective domains. Our incomplete understanding of the pathophysiological mechanisms underlying this process contributes to the partial comprehension of its potential clinical expression. This review focuses on the proposed pathophysiological processes underlying disease recrudescence in RA and the variable definitions adopted to capture flare in clinical practice through its objective, subjective, and temporal domains. Overall, what emerges is a complex landscape far from being unraveled.

Keywords: rheumatoid arthritis, flare, pathophysiology, clinical outcomes, composite disease activity indices

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INTRODUCTION

Identifying a pathological change in systemic chronic immune-inflammatory diseases is key to effective treatment strategies. In this context, one of the most compelling issues is the concept of flare. Whilst this is an easily comprehensible disease dynamic in theoretical terms, its omnicomprehensive definition still faces several challenges in clinical practice. If, on the one hand, these challenges may derive from incomplete knowledge of pathogenic dynamics linked to clinical manifestations, on the other hand, they derive from the multiple domains that characterize the constructs "disease activity." These include biological, subclinical, and subjective aspects of the pathology that might not be immediately measurable, not necessarily redundant, and whose relevance in the definition of the disease has been the object of continuous evolution.

A paradigmatic example of these concepts is rheumatoid arthritis (RA), which is frequently characterized by fluctuations in objective disease activity and subjective disease perception. RA hallmark characteristics had been identified, described, and collected within classification criteria by the ACR 1987 criteria (1). At that time, swollen joints only were considered pivotal in delineating the disease. Later, the EULAR/ACR 2010 criteria introduced tender joints as major defining findings of RA, recognizing elicited pain as a milestone characteristic of arthritis (2). Further, in the past few years, we have witnessed a breakthrough in the patient perspective, in the form of the so-called "patients reported outcomes," gaining an increasing role in defining the disease itself and the goodness of treatment's response. From this short timeline, it is notable how the complexity of RA had progressively been unveiled. It has been recognized that RA embraces more subtle and not

promptly measurable subjective domains besides the straightforward objective domains, all converging in the definition of the disease activity.

Due to the challenges discussed in the above paragraphs, numerous proxy definitions of RA flare (definable from a basic perspective, as the re-expression or enhanced expression of the disease pathogenic process) have been used in literature in the past years, according to the historical moment, the clinical context and the investigator's choice (**Table 1**).

In this narrative review, we will provide an updated overview of the concept of flare in RA, focusing on the most recent studies exploring this process from a pathobiological perspective. We will then discuss the two main perspectives that have been currently pursued to translate the process into clinically applicable definitions: (1) perspectives based on composite indices, firmly rooted into the objective domains of the disease through pre-set algebraic thresholds, (2) perspectives based on patients or clinician judgment, thus primarily based on the subjective perception of the process and overcoming the intrinsic limit of composite indices sometimes at the expense of a lower standardization.

PATHOPHYSIOLOGY OF RA FLARE

Although the tissue and immune processes supporting active RA have been thoroughly investigated (44, 45), insights into the pathodynamics of remission and flare remain scarce. Currently, it is unclear whether the transition from arthritis remission to flare recapitulates the events involved in arthritis onset or whether the process is driven by different mechanisms in post-injured joints (**Figure 1**). Supporting the rationale of this question, various studies suggest the possibility that the remission status, rather than being a simple restitutio ad integrum, might be characterized by specific patho-biologic changes, including active processes in which the pathology is kept in check by regulatory mechanisms (balanced homeostasis) and inflammation memory traits.

Gene transcriptional profiling of peripheral blood mononuclear cells of children with polyarticular juvenile idiopathic arthritis in remission failed to demonstrate a return to normalcy, highlighting persistency of pro-inflammatory and anti-inflammatory genes networks, apparently keeping the pathologic process in balance (46). From a more peripheral (articular) perspective, evidence from experimental models and RA patients in remission demonstrated that arthritis resolution could be mechanistically based on enhanced induction of type 2 innate lymphoid cells (ILC2) by IL-9, which in turn elicits the activation of regulatory T cells (47). Resident eosinophils, consistently present in the synovia of patients with RA in remission, have been shown to promote arthritis resolution by secreting resolvins and switching synovial macrophages into the M2 phenotype (48). Only recently, Alivernini et al. (49) provided further evidence supporting the concept of active remission. A specific subset of tissue-resident macrophage has been identified in remission RA patients' synovia. In particular, MerTK positive, CD206 positive synovial tissue macrophages (STM) are up-regulated in the synovia of RA patients in remission with respect to active RA. These STM produce anti-inflammatory and resolving molecules acting on synovial fibroblasts and into the joint milieu, actively supporting the maintenance of joint homeostasis.

Focusing directly on the process of flare, Kuettel et al. explored the longitudinal associations between patient-reported flares and inflammatory dynamics on MRI. Pointing to an "outside-in" hypothesis, sequential analysis of inflammatory imaging changes in the hands showed a differential lesion pattern: synovitis and tenosynovitis increased early at flare onset, while bone marrow oedema evolved with delay and remained present for months (32). Two independent fascinating studies have recently pioneered in this specific RA phase through immune-pathologic analysis. Based on a longitudinal follow-up of patients with RA with sequential evaluations together with single-cell RNA sequencing of blood cells, Orange et al. (26) could identify a population of mesenchymal cells (PRIME, preinflammatory mesenchymal cells) exhibiting an increase in circulation just before flares of RA, but decreasing just after the appearance of symptoms. The expression analysis of PRIME cells identified a profile similar to that of synovial inflammatory sublining fibroblasts, suggesting a model involving the active migration of these cells into the flaring joint and their causative contribution to local inflammatory events. Chang et al. through the analysis of three different animal models of arthritis, have shown the possible long-term persistence of synovial resident memory T cells (Trm) in arthritic joints during remission. The same authors could also demonstrate the central role of CD8+ Trm in the maintenance of joint-specific memory in quiescent joints and in the mechanism of recurrent joint-specific flares (50). Whilst these data provide a potential immune-biologic explanation of the known trend of arthritis flare to recur preferentially in previously involved districts in human disease (51, 52), the mechanistic link between Trm activation in the joint and local homing of circulating PRIME cells remains to be clarified.

Beyond the systemic and "synoviocentric" perspectives on the pathology of RA flare, a particular emphasis has been given to the mechanisms of defective drainage and lymphatic flow (53). Evidence derived from elegant studies in the murine system and RA using indocyanine green dye and direct near-infrared imaging has actually shown the existence of potential defects in the exit process associated with active disease in flaring joints, a mechanism that expands the anatomical substrate potentially involved in the event of RA disease recurrence (54–56).

CLINICAL TRANSLATION OF THE CONCEPT OF FLARE: COMPOSITE DISEASE ACTIVITY INDICES-BASED FLARE

Due to the lack of valid mechanistic biomarkers of flare, the goal of defining the process in clinical practice through quantitative and reliable approaches has led to the attempt to identify specific threshold adapting conventional indices

TABLE 1 | Rheumatoid arthritis flare definitions.

Composite disease activity score-based flare		
ΔDAS28 > 1.2 or > 0.6 if the final DAS28 ≥ 3.2		(3–5, 8)
ΔDAS28 > 1.2 or >0.6 if the initial DAS28 ≥ 3.2		(6, 7)
ΔDAS28 ≥ 1.2 or ≥ 0.6 if the initial DAS28 ≤ 3.2		(9)
ΔDAS28 > 0.6 and DAS28 > 2.6		(10)
ΔDAS28 > 1.2 or >0.6 if current DAS28 > 5.1		(11–13)
ΔDAS28 ≥ 0.6 and DAS28 > 3.2		(14)
ΔDAS28 > 1.2		(15)
ΔDAS ≥ 0.6 and DAS > 2.4 (any baseline DAS) or DAS > 2.4 from a previous DAS ≤ 2.4		(16)
DAS28-CRP ≥ 2.4		(17)
DAS28 > 3.2		(18, 19)
DAS28 > 2.6		(20)
DAS ≥ 1.6		(21)
DAS > 2.4 and/or SJC > 1		(22, 23)
Patient-based flare		
Δ ≥ 4.8-points in SF36-Bodily Pain score		(9)
RA Flare Questionnaire (no thresholds defined yet)		(7)
FLARE-RA questionnaire < 2.3 (overall), <1.8 (arthritis subscale), and <3.8 (general symptoms subsale) rules out flare		(24, 25)
RAPID3 score > 2 SD above the baseline mean		(26)
RAPID3 > 4.27 (physician judgment) and > 4.33 (patient judgment)		(27)
RADAI5 > 4.5 (physician judgment) and > 4.7 (patient judgment)		(27)
"Over the last 3 months, did you experience symptoms suggestive of disease exacerbation?"		(10)
Complex clusterings of intense, unprovoked symptoms that defy self-management (not necessarily captured in joint counts or global VAS) that lead the patient to seek help		(28)
"During the past 6 months, have you had a flare in your rheumatoid arthritis?"		(29)
"Have you had any episode/episodes of tender and swollen joints?"		(30)
"Has your disease flared up since the last assessment?"		(31)
"Are you experiencing a flare of your RA at this time?" with a possible rating of severity and duration		(6, 7, 32)
Physician-reported flare		
Worsening of disease activity that required treatment beyond the permitted therapy based on investigator opinions		(33)
Worsening of signs and symptoms of sufficient intensity and duration to lead to a change in therapy		(34)
Any worsening of disease activity leading to initiation/change/increase of therapy or an expression such as "flare up," "ongoing," and "active" in the medical records		(35)
Recurrence of synovitis such that discontinuation of the protocol was considered necessary		(36, 37)
Investigator judgment of poorly tolerated flare		(38)
Doctor's intention to treat		(39)
Combined flare definitions		
Objective	**Subjective**	
CDAI score > 10 or	Investigator's judgment of flare	(40)
ΔDAS28 ≥ 1.2 or ≥ 0.6 if final DAS28>3.2 OR	Investigator's judgment of flare	(41)
DAS28> 2.6 or inflammatory signs or	Inflammatory symptoms	(42)
Two of the following three: ΔDAS28 ≥ 1.2 and/or doubling of TJC and SJC and/or	Investigator's judgment of flare	(43)

DAS28, disease activity score on 28 joints; CRP, C-reactive protein; DAS, disease activity score; SJC, swollen joint count; SF-36, 36-Item Short Form Survey; RA, rheumatoid arthritis; RAPID3, routine assessment of patient index data 3; SD, standard deviation; RADAI5, Rheumatoid Arthritis Disease Activity Index-Five; VAS, visual analog scale; CDAI, clinical disease activity index; TJC, tender joint count.

of disease activity. Disease activity scores are widely used in clinical practice and as outcome measures in randomized clinical trials. All of them are built to embrace some of the most relevant defining aspects of RA activity, spanning through objective, subjective, and laboratory domains. However, their use and sensitivity to change have been validated limited to the improvement of the state of the disease, whether this was defined as achieving a designated level of relative improvement from baseline (American College of Rheumatology responses) (57), or as an improvement from baseline and specific state of activity of the disease in absolute terms [European League Against Rheumatism (EULAR) responses criteria] (58).

The OMERACT RA Flare workgroup provided a first working definition of flare in 2009: "A flare occurs with any worsening

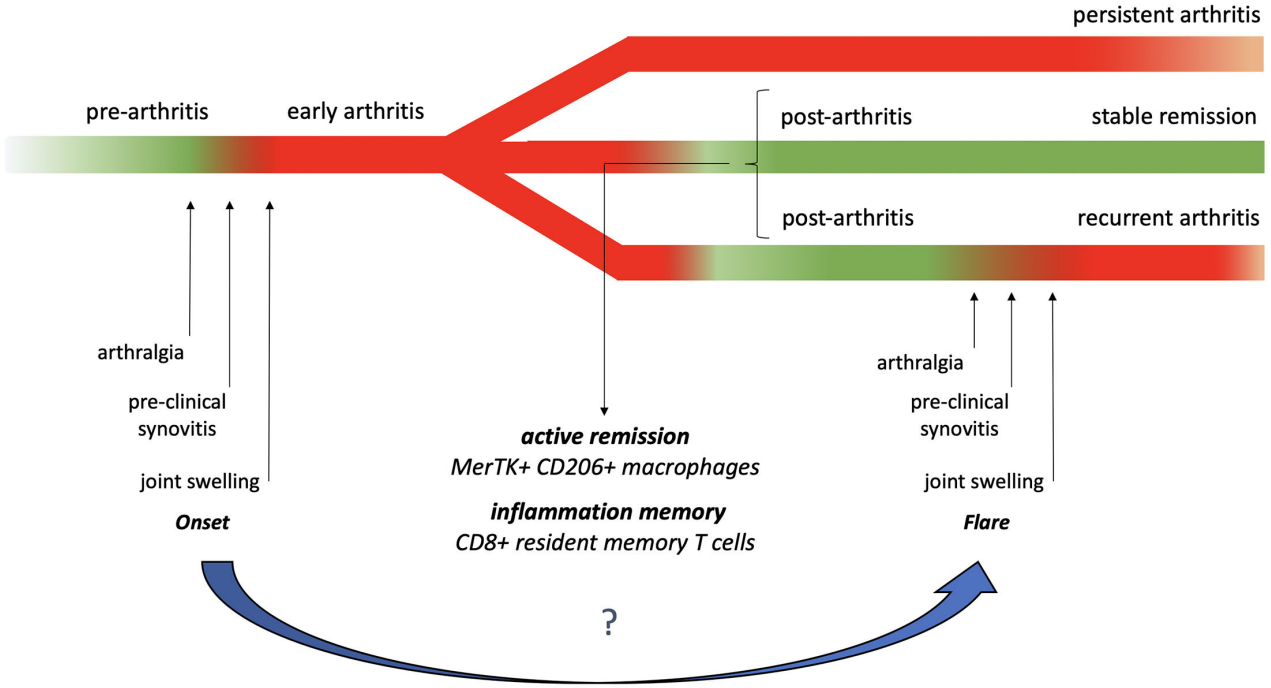

FIGURE 1 | Rheumatoid arthritis activity natural history. Rheumatoid arthritis's natural history starts with pre-arthritis, where immunological and inflammatory changes occur in the absence of overt clinical manifestations. Following this phase, the process can evolve in overt joint swelling and in the typical clinical manifestations of RA. Patients may then experience a persistent state of active disease, reach and maintain remission, or experience a flare where a stepwise process resembling onset may occur. The mechanisms supporting the homeostasis in the post-arthritis remissions phase and the biologic dynamics of flare have not been fully clarified yet. The green and red sections of the diagram represent phases characterized by the absence or the presence of joint clinical manifestations, respectively.

of disease activity that would, if persistent, in most cases lead to initiation or change of therapy; and a flare represents a cluster of symptoms of sufficient duration and intensity to require initiation, change or increase in therapy" (34). In 2013, van der Maas et al. tackled a more precise standardization providing specific thresholds to define minimal significant deviation capable of proving a substantial deterioration of the disease state, such as being called a flare. The proposed definition of flare is based on the DAS28 score and was obtained from the analysis of construct and criterion validity of previously proposed thresholds and minimal significant variations (3, 4, 11, 12, 15, 18, 59–61). DAS28-based flare is defined as DAS28 variation between two subsequent visits (Visit 1 and Visit 2) of more than 1.2 or more than 0.6 if the DAS28 was higher than or equal to 3.2 at the final visit (Visit 2) (sensitivity 63–78% and specificity 84–92%, using questions on disease activity worsening completed by patients and physicians as a gold standard's proxy) (5).

Before that, several unvalidated RA flare definitions have been used in clinical studies or proposed in the literature. These criteria vary considerably, ranging from physician-reported worsening to specific levels of change in core set variables or necessity to modify treatment (**Table 1**). However, the use of composite disease activity indices other than DAS28 in trials is limited to the work of Asai et al. (40), in which CDAI > 10 was used to define flare. Notably, an absolute value of DAS28 alone was frequently used when assessing flare in remission patients, where a variation of DAS28 may overestimate RA flare in patients fluctuating within the limits of remission (17, 19–22, 42). In some of these studies, to overcome the possibility of DAS28-based flare driven by subjective domains in the absence of objective synovitis, the additional or alternative presence of swollen joints was required (5, 21, 22, 61, 62). Indeed, it was observed that DAS28-based flare occurs more often than investigator-defined flare (1.7–7.3 times higher) (33) and, on the other hand, may miss patient-reported flare (PRF) (6). For this reason frequently the absolute threshold or minimal change in composite disease activity scores required to define flare were associated with or could be overcome by the investigator's judgment of flare (40, 41, 43).

Collectively, these data emphasize the potential limitations of the strict application of composite disease activity indices for the definition of flare in clinical practice (in particular in real life settings) and the relevance of the ongoing work of OMERACT to identify, define and standardize new domains, mainly based on patients' perception, to increase sensitivity and specificity of flare definition (34).

CLINICAL TRANSLATION OF THE CONCEPT OF FLARE: PATIENT-BASED AND PHYSICIAN-REPORTED FLARE

The physician judgment or the necessity of treatment modification has been applied in various works as the solely

possible definition of flare (33, 34, 39). The rheumatologist's view can be actually considered as the only comprehensive tool to integrate information derived from the objective and laboratory parameters with the patient's perspective through the filter of an expert interpretation. The discordance between subjective and DAS28-based definitions of flare, as mentioned above, is however complicated by further discordance in different subjective definitions themselves. Indeed the agreement between patient-based and physician-reported flare (similarly to the agreement between patient- and DAS28-based flare) is significantly affected by the degree of disease activity. Among patients starting from a DAS28-defined remission status, a high agreement (κ's $\geq$ 0.73) was observed. In contrast, a progressively reducing agreement was observed in patients starting in low disease activity (κ's = 0.44) and moderate-high disease activity (κ's = 0.21–0.35) (6). This observation is well-reflected by the numerous subjective definitions of flare reported in the literature (**Table 1**).

Various complex validated questionnaires have been produced to assess patient-relevant domains. The OMERACT RA Flare workgroup recently validated the Rheumatoid Arthritis Flare Questionnaire (RA-FQ) (7) which encompasses pain, physical impairment, fatigue, stiffness, and participation, including those relevant domains identified in previous OMERACT works (63) and not covered by both Routine Assessment of Patient Index Data 3 (RAPID3) (64) and the Rheumatoid Arthritis Disease Activity Index-Five (RADAI5) (64); however, appropriate thresholds for determining RA flares have not yet been established. Differently, for RAPID3 and RADAI5, two broadly used self-report questionnaires in everyday practice both in the US and in Europe, cutoffs to identify flare based on physician and patient-reported perspectives have been proposed: 4.27 and 4.33 for RAPID3 and 4.5 and 4.7 for RADAI5, respectively (27).

The French-born FLARE-RA questionnaire is another possible tool to help the physician recognize flare from the patient's perspective (65). It has proved itself to be able to identify patients with fluctuating disease activity, especially in those patients with low disease activity or remission. Different cutoffs recognized to have good sensitivity and specificity have been proposed. Myasoedova et al. (24) identified a lower (for clinical detection) and upper (for therapeutic change) cutoff varying depending on the duration of disease. More recently, Aouad et al. (25) identified a clearer cutoff of 2.3 for the FLARE-RA general score, able to detect a patient "in flare" (above) vs. "not in flare" (below) over the past 3 months or since the last visit.

Modification of disease activity in RA is strongly related to pain, a subjective domain concretized with visual analog or numerical rating scales (9). Pain perception can be associated with tender joints or exist without elicitable joint pain. Indeed, pain has a complex biological background. The time spent in a chronic inflammatory state, such as RA, can affect different levels of the signaling cascade that modify perception and thresholds for pain (66, 67). Frequently, arthralgia is the first manifestation of RA even in the absence of objective synovial inflammatory processes or tender joints, like in pre-clinical arthralgia phases (68). Similarly, isolated arthralgia episodes frequently occur during the natural history of the disease and might be transient or prelude to recrudescence. Overall, the presence of pain, despite physician judgment, is a relevant domain identified by patients when defining flare. When we approach patient-based definitions of flare, it is critical to keep in mind that patient perception is partially modified by time spent with the disease. Experienced patients suggested that the longer you lived with the disease, the better you are at placing a worsening within the context of disease variability and not worrying about a flare (34).

Of interest, McWilliams et al. (9) recently proposed a new flare entity based primarily on pain and assessed by the SF36-bodily pain scale. They identified patients experiencing abrupt (primary) or progressive (incremental) pain flares as suggested by a minimal predetermined variation in the SF36-bodily pain scale. These exacerbations were discordant with DAS28 flare in 23 and 70% of cases, respectively. Despite a significant discordance rate between the pain and DAS28-based flare definitions, both were associated with a persistent increase in disability even after flare improvement.

Apart from validated questionnaires and scores, there are many domains not yet addressed by current assessment scales, which patients nevertheless recognize as essential aspects of disease activity. The intrinsic difficulty in measuring subjective domains has led many authors to evaluate the presence or absence of flare based on a simple anchor question considering the overall patient perspective: "have you experienced a flare since your last visit?" (66). This broad question provides an overarching summary of all the information that we miss to measure and that the patient recognizes as red flags of disease deterioration or recrudescence.

DURATION OF FLARE

Duration is one of the critical aspects that must be tackled to provide a solid definition of flare. Indeed, some exacerbations are short-lived (a "bad day") and often managed with rest or non-pharmacological interventions. Others, more severe, may require clinical intervention (34). Fluctuation in disease activity is expected in the natural wax and weaning history of RA, and spontaneous resolution of a transient deterioration may be expected. Thus, differentiating flare from physiological fluctuations could avoid overtreatment strategies, which could be partially achieved by better understating the timing of flare.

Although the current criteria and definitions did not tackle systematically flare duration, some authors have addressed this point to better characterize flare.

The length of time spent by the patient in a flare state may span from a few days to several weeks in the current literature. Jacquemin et al. (31) reported that 79% of self-assessed flares were short flares (<3 days), while the remaining were persistent flares (more than 3 days) (31). The AMBRA trial (30) differentiated between transient reported flares (<14 days) and constant joint complaints when lasting for at least 1 year. In the DRESS study instead (8), the persistence of significant symptoms deterioration for more than 12 weeks was addressed as major flare, while shorter symptoms were considered short

flares. The concept of time spent on flares, together with their frequency, is of tremendous importance. In fact, the length and frequency of flares are associated with radiographic progression and deterioration of physical function, an increase in CVD risk of 7% for each flare (considered to last 6 weeks), and a reduction in physical activity by a median of 1000 steps per day of flare, as recorded by connective activity trackers (31, 32, 35).

CONCLUSION

The concept of flare in rheumatoid arthritis is blurred. The difficulty lies in the complexity of the multifaceted manifestations of rheumatoid arthritis, where subjective and objective domains converge in determining disease activity. The current criteria for the definition of flare are the first important step toward a better characterization to facilitate the recognition of this event in clinical research and trials. However, they still appear to lack those desirable omni-comprehensive capabilities for the routing application in real-life clinical practice. Challenges in this direction may derive not only from the intrinsic multi-dimensional nature of RA disease activity in individual patients but also from the potential heterogeneity of flare in different individuals. In particular, the predictable dynamic nature of the process of disease flare in RA might progress through various stages characterized by different expressiveness of objective and subjective domains (as in the case of the transition between pre-clinical and overt RA). The heterogeneous phatophysiological substrate of RA may delineate differences in the clinical expressiveness of flare in different disease subsets (for example, ACPA positive and ACPA negative RA). Finally, the objective and subjective expression of the flare process might be characterized by specific differences depending on the phase of the disease or its treatment protocol (early, late-stage, under treatment, or under drug-free conditions).

Collectively, this review points to the need for further research in this direction, a fundamental area of investigation that could turn out to be essential for improving patient monitoring, for the definition of new therapeutic targets, and for a deeper understanding of the pathophysiology of RA.

AUTHOR CONTRIBUTIONS

EB-C, SG, BX, TL, MG, IM, SB, CM, and AM contributed to the literature review and manuscript drafting. All authors contributed to the article and approved the submitted version.

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REVIEW
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B Cells on the Stage of Inflammation in Juvenile Idiopathic Arthritis: Leading or Supporting Actors in Disease Pathogenesis?

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Juvenile idiopathic arthritis (JIA) is a term that collectively refers to a group of chronic childhood arthritides, which together constitute the most common rheumatic condition in children. The International League of Associations for Rheumatology (ILAR) criteria define seven categories of JIA: oligoarticular, polyarticular rheumatoid factor (RF) negative (RF-), polyarticular RF positive (RF+), systemic, enthesitis-related arthritis, psoriatic arthritis, and undifferentiated arthritis. The ILAR classification includes persistent and extended oligoarthritis as subcategories of oligoarticular JIA, but not as distinct categories. JIA is characterized by a chronic inflammatory process affecting the synovia that begins before the age of 16 and persists at least 6 weeks. If not treated, JIA can cause significant disability and loss of quality of life. Treatment of JIA is adjusted according to the severity of the disease as combinations of non-steroidal anti-inflammatory drugs (NSAIDs), synthetic and/ or biological disease modifying anti-rheumatic drugs (DMARDs). Although the disease etiology is unknown, disturbances in innate and adaptive immune responses have been implicated in JIA development. B cells may have important roles in JIA pathogenesis through autoantibody production, antigen presentation, cytokine release and/ or T cell activation. The study of B cells has not been extensively explored in JIA, but evidence from the literature suggests that B cells might have indeed a relevant role in JIA pathophysiology. The detection of autoantibodies such as antinuclear antibodies (ANA), RF and anti-citrullinated protein antibodies (ACPA) in JIA patients supports a breakdown in B cell tolerance. Furthermore, alterations in B cell subpopulations have been documented in peripheral blood and synovial fluid from JIA patients. In fact, altered B cell homeostasis, B cell differentiation and B cell hyperactivity have been described in JIA. Of note, B cell depletion therapy with rituximab has been shown to be an effective and well-tolerated treatment in children with JIA, which further supports B cell intervention in disease development.

Keywords: juvenile idiopathic arthritis (JIA), B cells, autoantibodies, inflammation, immunopathogenesis

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INTRODUCTION

B cells have several important roles in autoimmunity such as autoantibody production, antigen presentation, cytokine release, and T cell activation. B cell development originates in the bone marrow, where these cells undergo different maturation stages and critical checkpoint mechanisms to ensure tolerance and are released in the periphery as immature cells. These antigen-naïve B cells circulate through the blood and lymphatic systems to secondary lymphoid organs where, upon activation and exposure to antigen, they proliferate and differentiate into memory B cells or antibody-producing plasma cells. During B cell differentiation process at germinal centers, B cells are clonally expanded and go through somatic hypermutation, affinity maturation and class or isotype switching (**Figure 1**) (1). Defects in central tolerance mechanisms (clonal deletion, anergy, and/ or receptor editing) occurring in the bone marrow and/ or during peripheral tolerance can contribute to the development of autoreactive B cells and autoimmune diseases (2–4). Juvenile idiopathic arthritis (JIA) is the most common rheumatic disorder in children. Disturbances in both innate and adaptive immune systems have been described in JIA patients. The study of B cells has not been extensively explored in JIA, but evidence from the literature suggests that B cells might have a relevant role in JIA pathogenesis (5–7). Notably, the detection of autoantibodies such as antinuclear antibodies (ANA), rheumatoid factor (RF), and anti-citrullinated protein antibodies (ACPA) in JIA patients supports a breakdown in B cell tolerance (8). Furthermore, it has been shown that JIA patients have increased rates of secondary V(D)J recombination (normally restricted to early B-cell precursors in the bone marrow) in peripheral blood B cells, with a skewed kappa (κ):lambda (λ) light chain usage (9–11). These data suggest that mature peripheral blood B cells of JIA patients have the potential to perform receptor revision outside the bone marrow and, therefore, promote autoimmunity (9–11). In addition, altered B cell homeostasis, B cell differentiation, and B cell hyperactivity have been described in JIA, which further supports B cell intervention in disease development (12–19).

JUVENILE IDIOPATHIC ARTHRITIS: DEFINITION, CLASSIFICATION AND DISEASE CATEGORIES

Juvenile idiopathic arthritis (JIA) is a term used to classify a group of heterogeneous chronic childhood inflammatory arthritides of unknown etiology, which together constitute the most common rheumatic condition in children (20). JIA is characterized by a chronic inflammatory process affecting the synovia that begins before the age of 16 and persists at least 6 weeks. Nonetheless, in some children, JIA can be a lifelong condition. JIA affects not only joints, but also extra-articular structures, including eyes, skin, and internal organs and, if not treated, can lead to serious disability and loss of quality of life (21, 22). The International League of Associations for Rheumatology (ILAR) criteria define seven categories of JIA: oligoarticular, polyarticular RF negative (RF-), polyarticular RF positive (RF+), systemic, enthesitis-related arthritis, psoriatic arthritis, and undifferentiated arthritis (23). The ILAR classification includes persistent and extended oligoarthritis as subcategories of oligoarticular JIA, but not as distinct categories. JIA initial classification is determined according to the clinical features presented during the first 6 months of disease course, such as the number of affected joints, severity of disease, and presence or absence of inflammation in other parts of the body. The onset of new clinical features during the course of the disease determines the final disease subtype.

Oligoarticular

Oligoarticular JIA (oJIA) is the most common JIA subtype. It is defined as asymmetric arthritis affecting up to four joints, mainly large joints such as the knee, ankle, wrist, and/ or elbow, during the first 6 months after disease onset (23). oJIA can be subcategorized in two types of arthritis: *persistent oJIA* (no more than four joints are affected during the course of the disease) and *extended oJIA* (more than four joints are affected after the first 6 months of disease). oJIA usually begins before 4 years of age and female gender is predominantly affected (24). ANA are present in up to 60–80% of patients with oJIA. Importantly, oJIA is associated with a high risk of uveitis (24, 25), particularly in ANA positive (ANA+) patients (26–28).

Polyarticular

Polyarticular JIA (pJIA) is the second most common JIA subtype, defined as arthritis in which 5 or more joints are affected during the first 6 months of the disease. There are two forms of pJIA: polyarticular RF negative (pJIA RF-) and polyarticular RF positive (pJIA RF+) (23). Female gender is predominant in both types of pJIA.

Polyarticular Rheumatoid Factor Negative

Patients with pJIA RF- have mostly asymmetric arthritis of small and large joints, and disease onset usually occurs between 2 and 12 years old. ANA can be detected in up to 40% of pJIA patients and uveitis can be present in up to 10% of cases (23–25).

Polyarticular Rheumatoid Factor Positive

Patients with pJIA RF+ have symmetric arthritis mainly of small joints (metacarpophalangeal joints and wrists) and disease onset is more frequent in late childhood or adolescence. Arthritis development is associated to a more erosive and aggressive disease progression (23–25).

Systemic

Systemic JIA (sJIA) is a disease subtype defined by the presence of arthritis in one or more joints and concomitant systemic manifestations that include fever persisting for more than 2 weeks, generalized lymphadenopathy, rash, hepatosplenomegaly, and/ or serositis (23). Disease onset may occur at any time during childhood and both female and male genders are equally affected. Autoantibodies are usually absent (25, 29). A dysregulation of the innate immune system has been associated to the systemic inflammation present in sJIA, suggesting that this JIA subtype may rather be part of the spectrum of autoinflammatory

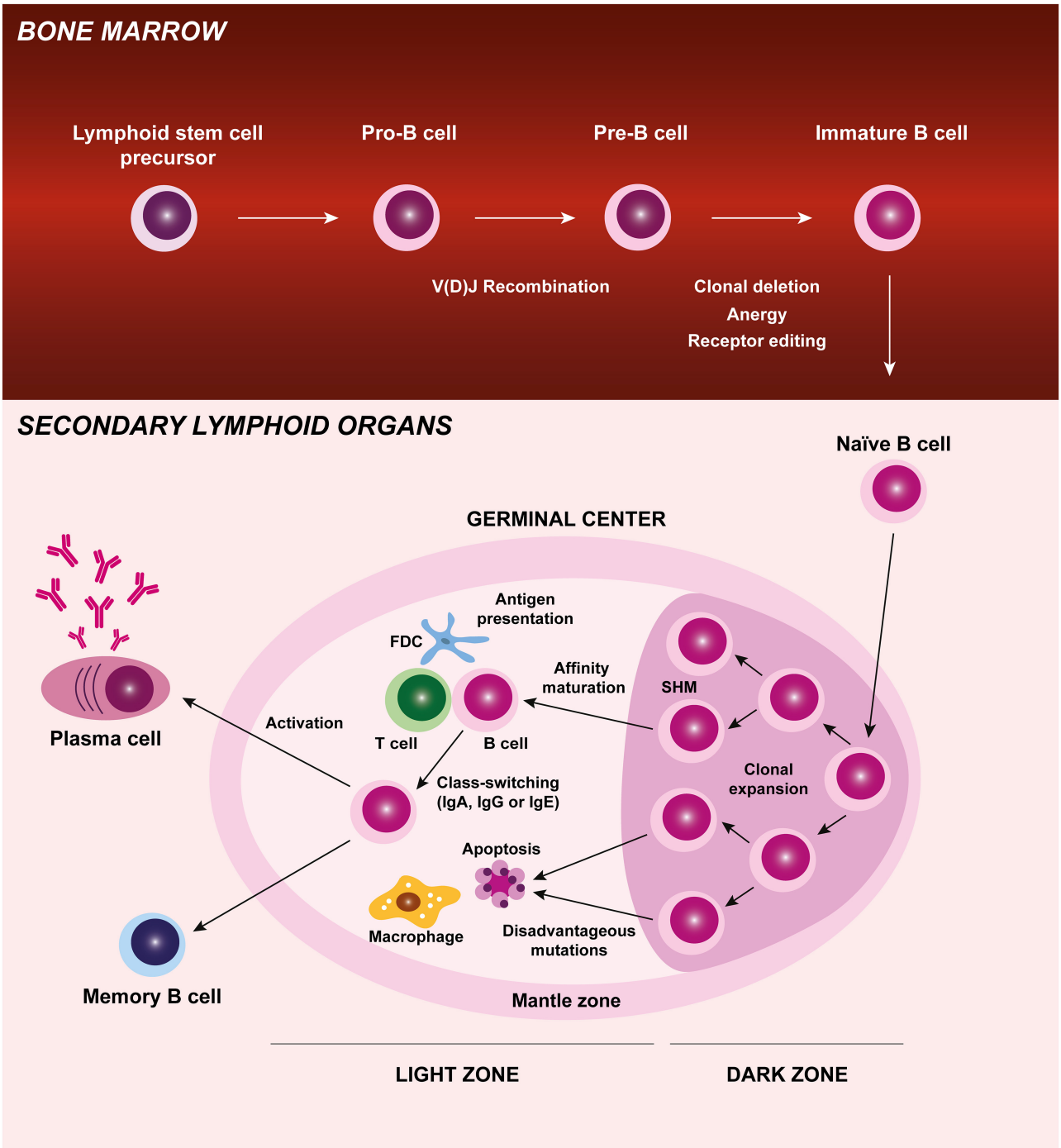

FIGURE 1 | B cell origin and development. B cells originate from a lymphoid stem cell in the bone marrow and proceed through several maturation stages, during which V(D)J recombination and central tolerance mechanisms (clonal deletion, anergy, and receptor editing) occur. B cells leave the bone marrow and are released in the periphery as immature cells. These antigen-naïve B cells circulate through the blood and lymphatic systems to secondary lymphoid organs (lymph nodes, spleen, and Peyer's Patches) where, upon activation and exposure to antigen, they proliferate and differentiate into memory B cells or antibody-producing plasma cells. During B cell differentiation process at germinal centers, B cells are clonally expanded and go through somatic hypermutation, affinity maturation, and class-switching. Autoreactive B cells that may develop during B cell differentiation process and recognize self-antigens are eliminated by apoptosis. FDC, follicular dendritic cell; Ig, immunoglobulin; SHM, somatic hypermutation.

disorders (29–33). Nevertheless, alterations in adaptive immunity have also been described in sJIA (34–38).

Enthesitis-Related Arthritis

Enthesitis-related arthritis (ERA) is a subtype of JIA that affects the joints of the lower limbs (hip, knee, ankle, and foot) in association with enthesitis. In addition, axial involvement and arthritis of the sacroiliac joints and upper limbs, particularly the shoulders, can also occur (23). ERA is more frequent in male gender. Disease onset usually occurs in late childhood or adolescence. Acute anterior uveitis and gut inflammation can also be present in ERA patients. The diagnosis of this JIA subtype is strongly associated to the major histocompatibility complex (MHC) class I antigen human leukocyte antigen (HLA)-B27 (25).

Psoriatic Arthritis

Juvenile psoriatic arthritis (JPsA) is characterized by an asymmetric arthritis of small and large joints and the presence either of a psoriatic rash or, in the absence of rash, at least two of the following criteria: first-degree relative with psoriasis, nail pitting or onycholysis, and dactylitis (23). Clinical symptoms may also include uveitis. JPsA is composed of two subgroups, differentiated by age at onset. Children with early-onset JPsA (<6 years old) are predominantly female, ANA+, more predisposed to uveitis, with arthritis of the wrists and small joints of the hands and feet. In contrast, children with later-onset JPsA are more associated to male gender, axial disease, enthesitis, and HLA-B27 positivity (39, 40).

Undifferentiated Arthritis

Undifferentiated juvenile idiopathic arthritis includes patients who do not fulfill the criteria for any JIA category above described, or who meet the criteria for more than one (23, 41).

The main clinical features of all JIA categories are summarized in **Table 1**. Notably, the complexity and heterogeneity of JIA diagnosis is still controversial and subject to new classification proposals (42, 43).

ETIOLOGY AND RISK FACTORS OF JUVENILE IDIOPATHIC ARTHRITIS

The cause of JIA is unknown. Nevertheless, JIA has been established as an autoimmune disorder in which genetic susceptibility and environmental factors are associated to disease development. JIA might be initially triggered by the exposure to environmental factors in children with a genetic predisposition to synovial inflammation. Infections, vaccines, antibiotics, vitamin D deficiency, stress, and trauma have been proposed as environmental risk factors for JIA progression (25, 44, 45). In fact, it has been reported that infectious viruses (Epstein-Barr virus, Parvovirus B, Rubivirus, and Hepatitis B virus) and bacteria (*Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Streptococcus pyogenes*, *Bartonella henselae*, *Mycoplasma pneumoniae*, and *Chlamydophila pneumonia*) may act as triggering agents of JIA (46). Furthermore, disturbances in the gut microbiome have been shown to increase the risk of JIA development (47–49). Additionally, evidence from the literature suggests that maternal smoking during pregnancy can also be a risk factor for JIA (44, 50). Genetic predisposition to JIA is mainly due to human leukocyte antigen (HLA) class II molecules, although HLA class I molecules and non-HLA genes have also been implicated, depending on the disease category (24, 25, 29, 51–57).

IMMUNOPATHOGENESIS OF JUVENILE IDIOPATHIC ARTHRITIS

The mechanisms of immunopathogenesis of JIA are still poorly understood. JIA categories are complex, heterogeneous, with different contributions of immune system players and effector cells (24, 25, 58–63). Indeed, several studies have demonstrated a predominance of adaptive immunity in the pathogenesis of oJIA, pJIA, ERA, and JPsA (14, 17, 18, 24, 25, 39, 57, 64–86), whereas innate immune responses are the major contributors to disease development and progression in sJIA (29, 59, 87–98). In fact, oJIA, pJIA, ERA, and JPsA are classified as autoimmune diseases, while sJIA has been proposed as an autoinflammatory disorder (25, 58, 59). In JIA, joint inflammation, swelling and tissue destruction are a hallmark of the disease. The pathophysiology mechanisms associated to JIA development are related to an abnormal activation of immune system cells such as B cells, T cells, natural killer (NK) cells, dendritic cells (DCs), monocytes, neutrophils, plasma cells, and to the production and release of pro-inflammatory mediators (cytokines, chemokines, enzymes such as matrix metalloproteinases, aggrecanases, and cathepsins) that ultimately lead to cartilage and bone destruction and systemic manifestations. The inflammatory process that occurs at the synovial joint leads to the thickening of the synovial membrane due to an excessive proliferation of synoviocytes and infiltration of the sub-lining layer of the synovium by immunocompetent cells (lymphocytes, macrophages, granulocytes, plasma cells…), which causes hyperplasia and hypertrophy of the synovium (**Figure 2**). Consequently, intra-articular hypoxia occurs and pathological angiogenesis initiates. The new blood vessels that form within the synovium increase blood supply and contribute to the migration of pro-inflammatory cells into the joint, thus forming a pathological synovium known as "pannus." Overall, the complex cellular networks and the release of inflammatory mediators that occur within JIA synovium stimulate chondrocytes and osteoclasts that trigger cartilage and bone erosion, respectively (**Figure 2**) (99–107).

B CELL ROLES IN JUVENILE IDIOPATHIC ARTHRITIS PATHOPHYSIOLOGY

JIA has been classically considered a T-cell driven autoimmune disease, except for sJIA subtype, in which innate immune cells have a central role in disease pathogenesis as previously mentioned. However, the detection of autoantibodies reacting with different target antigens in JIA patients suggests a central role of B cells in JIA pathophysiology. In fact, B cells may have important roles in JIA pathogenesis

TABLE 1 | Clinical features of juvenile idiopathic arthritis.

	JIA category					
	Oligoarticular	**Polyarticular RF−**	**Polyarticular RF+**	**ERA**	**JPsA**	**Systemic**
Clinical prevalence	50–60% of all JIA	10–30% of all JIA	5–10% of all JIA	10–15% of all JIA	5–6% of all JIA	10–20% of all JIA
Gender predominance	Female	Female	Female	Male	Female (early-onset) Male (later-onset)	Equal
Pattern of arthritis	≤4 joints affected; Mainly large joints; Asymmetric	≥5 joints affected; Small and large joints; Symmetric or asymmetric	≥5 joints affected; Mainly small joints; Aggressive symmetric polyarthritis; Erosive	Lower limb joints more commonly affected; Axial involvement; Sacroiliac joints; Upper limbs	Small and large joints; Asymmetric	≥1 joint affected; Mostly wrists, knees, ankles or asymptomatic temporomandibular arthritis
Systemic manifestations	Uveitis	Uveitis	Rheumatoid nodules; Uveitis	Acute anterior uveitis; Enthesitis; Gut inflammation	Psoriasis; Dactylitis; Onycholysis; Nail pitting; Uveitis	Fever; Generalized lymphadenopathy; Rash; Serositis; Hepatosplenomegaly; MAS
Autoantibodies	ANA	ANA, ACPA	ANA, RF, ACPA	ANA (some cases)	ANA (early-onset)	Usually absent
Disease biomarkers	60–80% ANA+	40% ANA+	RF+, ACPA+, 40% ANA+	45–85% HLA-B27+	50% ANA+	Increased levels of CRP, Ferritin, Leukocytosis, Thrombocytosis

ACPA, anti-citrullinated protein antibodies; ANA, antinuclear antibodies; CRP, C-reactive protein; ERA, enthesitis-related arthritis; HLA-B27, human leukocyte antigen-B27; JIA, juvenile idiopathic arthritis; JPsA, juvenile psoriatic arthritis; MAS, macrophage activation syndrome; RF, rheumatoid factor.

through autoantibody production, antigen presentation, cytokine release, and/ or T cell activation (**Figure 3**) (5–7). Although the study of B cells has not been extensively explored in JIA, evidence from the literature suggest the occurrence of alterations in B cell differentiation, homeostasis and hyperactivity in JIA patients, which can contribute to disease progression.

Autoantibody Production

Autoantibody production is a hallmark function of B cells in autoimmunity. In JIA, autoantibodies such as ANA, RF and ACPA can be detected in the serum of these patients (8, 108–114), which supports a breakdown in B cell tolerance. ANA are autoantibodies that can target several autoantigens within cell nucleus structures including nucleic acids, nucleosomes, phospholipids, and several nuclear and nucleolar proteins (115). These autoantigens, which are normally "hidden," are exposed to antigen presenting cells during cell death, particularly during apoptosis. ANA can be detected in several autoimmune diseases such as systemic lupus erythematosus (SLE), RA, Sjögren's syndrome, idiopathic thrombocytopenic purpura, mixed connective tissue disease, juvenile dermatomyositis, autoimmune hepatitis, primary biliary cirrhosis, ulcerative colitis, and autoimmune thyroiditis (116–125). In JIA patients, the overall seroprevalence of ANA among all subtypes of JIA combined is < 50% (126). ANA are more commonly detected in oJIA and pJIA (mostly in pJIA RF-) patients and are particularly more prevalent in young, female oJIA patients (127). In JPsA patients, ANA positivity is also more strongly associated with early-onset disease and female predominance (39). ANA are less commonly detected in sJIA and undifferentiated JIA (126). Although the exact contribution of ANA to JIA pathology remains unclear, previous reports have suggested that ANA positivity is associated with the development of ectopic lymphoid structures in synovial tissue (16). Interestingly, the presence of a lymphoid organization in synovial tissue from ANA+ JIA patients was strongly related to the concomitant degree of plasma cells infiltration (16). Thus, the development of these lymphoid structures could contribute to the interactions between autoreactive B and T cells, which could directly support the production of these autoantibodies and the inflammatory process in the joint. Furthermore, it has been demonstrated that ANA are associated to a higher risk of uveitis (119, 128). RF is an immunoglobulin of any isotype (predominantly IgM) that specifically recognizes the Fc portion of IgG molecules, which was first described in RA patients (129, 130) and is currently included in the classification criteria for RA (131). RF can be detected in other autoimmune disorders such as SLE, Sjögren's syndrome, systemic sclerosis, mixed connective tissue disease, polymyositis, dermatomyositis, as well as in healthy individuals (132, 133). RF have also been identified in JIA patients (8, 134) and, despite being present in a small subgroup of pJIA patients (only 5% of total JIA patients), RF positivity is associated with a worse disease prognosis (113, 135). Indeed, patients with pJIA

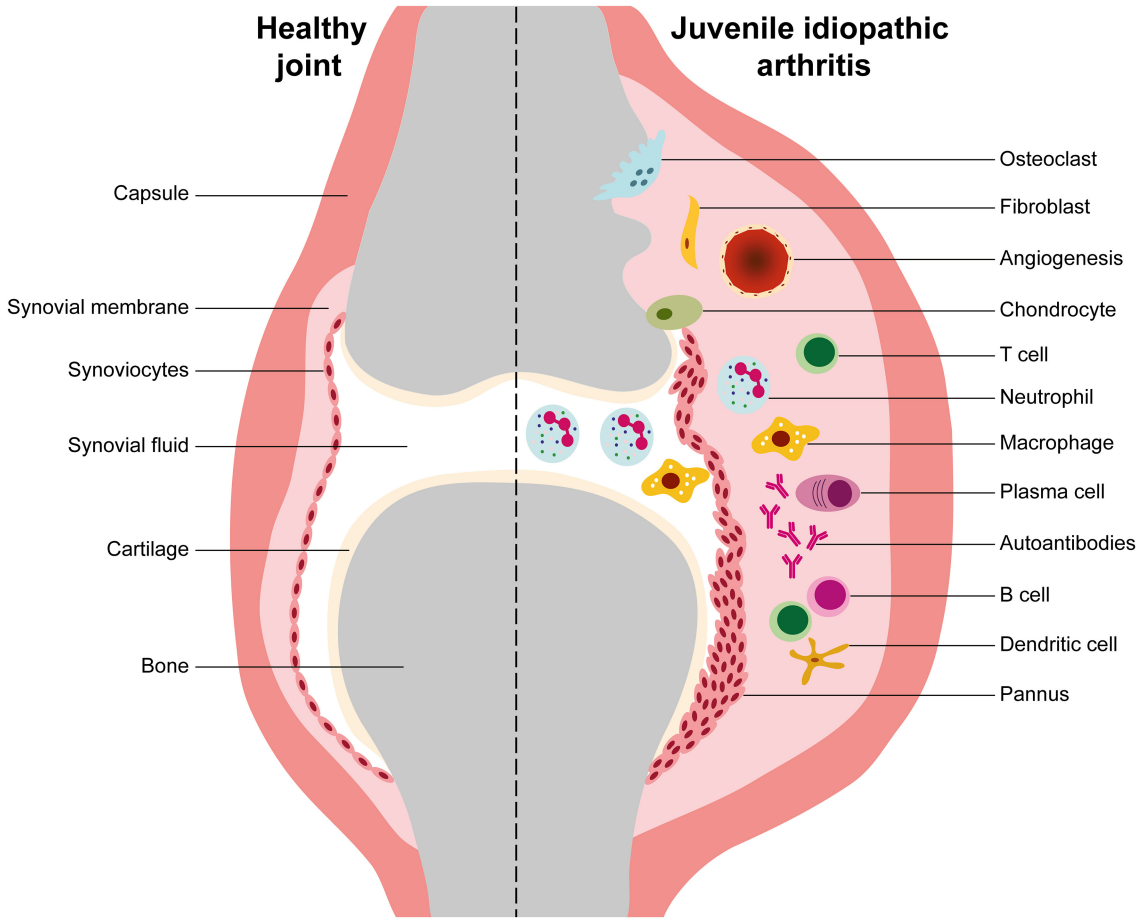

FIGURE 2 | Synovial inflammation in juvenile idiopathic arthritis. This is a representative scheme illustrating the differences between healthy and juvenile idiopathic arthritis (JIA) joints. In JIA, the inflammatory process that occurs at the synovial joint is characterized by an uncontrolled proliferation of synoviocytes and cellular infiltration of the sub-lining layer of the synovium by macrophages, granulocytes, B cells, T cells, and plasma cells, which causes synovial membrane hyperplasia. Consequently, intra-articular hypoxia occurs and pathological angiogenesis initiates. The new blood vessels that form within the synovium increase blood supply and contribute to the migration of pro-inflammatory cells into the joint, thus forming a pathological synovium known as "pannus." The complex cellular networks that occur within JIA synovium and the production of inflammatory mediators such as cytokines, chemokines and metalloproteinases stimulate chondrocytes and osteoclasts that trigger cartilage and bone erosion, respectively.

RF+ are at higher risk of a more aggressive disease course with cartilage and bone erosions than JIA patients without RF (113, 136–138). Notably, pJIA RF+ is considered the pediatric version of adult RA (139, 140). In fact, it has been demonstrated that the majority of pJIA patients, particularly pJIA RF+, evolve to RA in adulthood (21). Of note, these observations reinforce that pJIA RF+ and RA are likely to have similar underlying pathological mechanisms and that current treatment strategies applied in RA are directly relevant to pJIA RF+. RF can have important physiological roles in the normal immune system such as promoting phagocytosis and the removal of antigen-antibody complexes in the course of infection; fixation of complement; and enhancing B cell antigen uptake and presentation to CD4+ T cells (141). Nevertheless, these naturally-occurring RF are generally low-affinity and polyreactive, whereas pathogenic RF tend to have undergone affinity maturation (133, 142). Although the mechanisms of RF production are not entirely understood, previous reports have described to be dependent on immune-complex recognition by B cell receptors in the context of toll-like receptor stimulation, as well as T cell help (143–145). ACPA are autoantibodies that recognize citrullinated peptides and proteins and, similarly to RF, are included in the classification criteria for RA (131). Although ACPA are highly specific to RA diagnosis, these antibodies can also be detected in JIA patients, particularly in pJIA RF+ (111, 112, 114, 146–148). In fact, it has been demonstrated that ACPA detected in pJIA RF+ patients express the inherently autoreactive 9G4 idiotope, which supports an activation of autoreactive 9G4+ B cells in JIA (148), similarly to what has been described in RA patients (149). Importantly, ACPA detection in JIA patients is associated to more severe and erosive disease progression, which might implicate an earlier and more intensive treatment (8, 112–114, 146, 150, 151). During inflammation, ACPA might be produced in a process called citrullination, a post-translational modification catalyzed

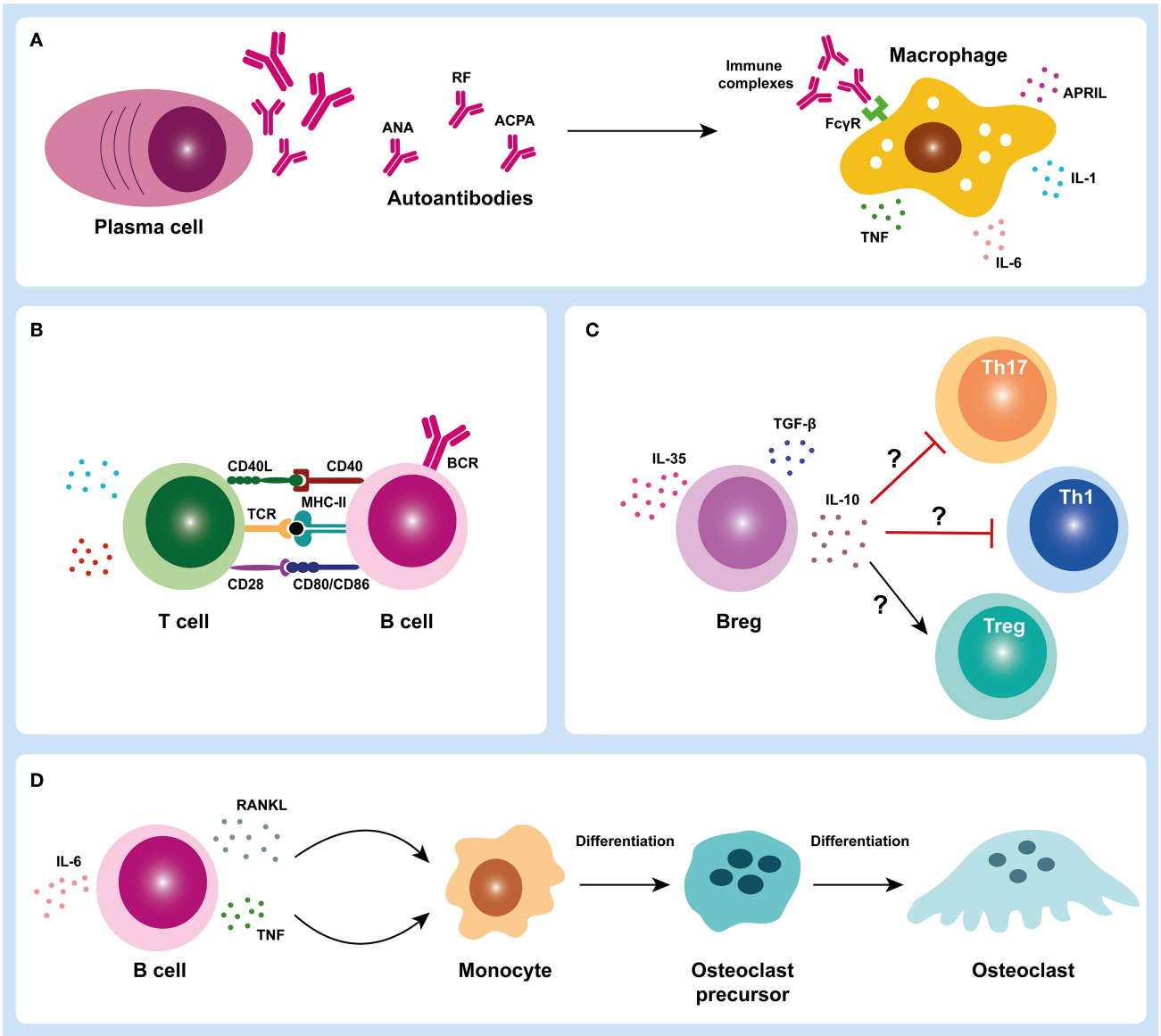

FIGURE 3 | B cell roles in juvenile idiopathic arthritis. B cells can have several important roles in juvenile idiopathic arthritis (JIA) pathogenesis through autoantibody production **(A)**, antigen presentation and/ or T cell activation **(B,C)**, cytokine release and contribute to bone damage in JIA synovial joint **(D)**. In JIA, activated B cells can differentiate into autoantibody-producing plasma cells. Plasma cells produce autoantibodies that can form immune complexes that deposit in the joints and trigger macrophage activation through Fc-gamma receptors (FcγR) **(A)**. Activated macrophages can release several pro-inflammatory mediators such as cytokines (IL-1, IL-6, and TNF) that contribute to the inflammatory process **(A)**. B cells can also act as efficient antigen presenting cells and activate T cells **(B)**. Regulatory B cells (Bregs) may also have an important contribution in JIA pathogenesis through either a defective suppression of T helper cell subsets (Th1 and Th17) and/ or an impairment of regulatory T cells (Tregs) activation **(C)**. B cells can also release cytokines such as TNF and RANKL that can activate osteoclastogenesis and trigger bone erosion in JIA synovial joint **(D)**. ACPA, anti-citrullinated protein antibodies; ANA, antinuclear antibodies; APRIL, A proliferation-inducing ligand; BCR, B cell receptor; Breg, regulatory B cell; CD, cluster of differentiation; FcγR, Fc-gamma receptor; IL, interleukin; MHC-II, major histocompatibility complex class II; RANKL, receptor activator of nuclear factor kappa-B ligand; RF, rheumatoid factor; TCR, T cell receptor; TGF-β, transforming growth factor beta; Th, T helper; TNF, tumor necrosis factor; Treg, regulatory T cell.

by peptidylarginine deiminases (PAD) enzymes in which arginine amino acid residues are enzymatically converted into citrulline residues in a wide range of proteins (152, 153). These structural changes in proteins can form new epitopes that trigger ACPA production by autoreactive B cells. Of note, both ACPA and RF autoantibodies are able to form immune complexes that deposit in the joints. These immune complexes can activate complement and macrophages through Fc-gamma receptors (FcγR) and induce cytokine release that contribute to the inflammatory process in JIA (**Figure 3A**) (8, 154, 155). Importantly, evidence

of hypergammaglobulinaemia correlated with clinical disease activity has been described in JIA patients (mainly in oJIA and pJIA), which is consistent with B cell hyperactivity (14).

Antigen Presentation, Cytokine Release, and T Cell Activation

During the inflammatory process in JIA, B cells can function as efficient antigen presenting cells and, once activated, can release cytokines and stimulate T cell activation, thus contributing to the exacerbation of inflammation (**Figure 3B**). A distinctive feature of chronic inflammatory arthritis is the presence of synovial lymphocytic infiltrates that play a role in disease pathogenesis by secretion of pro-inflammatory cytokines and other soluble mediators (156–166). Indeed, both B and T cells are detected in synovial infiltrates from JIA and RA patients (17, 18, 69, 79, 156, 163–165, 167–172). In particular, high levels of plasma cells infiltration have been detected in the synovial membrane of JIA patients (69, 173). Furthermore, it has been shown that lymphoid neogenesis in JIA is correlated to ANA positivity and plasma cells infiltration not only in synovia, but also in iris tissue, which further supports a dysregulated B cell activation in JIA patients (16, 174, 175). Notably, it was shown that JIA patients with ANA+ anterior uveitis often show an infiltrate of plasma cells in iris (174). In addition, it has been demonstrated that activated memory B cells accumulate in the inflamed joints of patients with JIA (17, 18, 171, 176). Of note, it was shown that class-switched CD27+ and CD27- memory B cells expressed up-regulated levels of the costimulatory molecules CD80 and CD86 and could activate allogenic CD4+ T cells *in vitro* more effectively when compared to peripheral blood B cells (18). Additionally, it was observed that synovial B cells could not only induce the activation and polarization of T helper (Th)-1 cells, but also secrete Th1-polarizing cytokines (18). Overall, the accumulation of memory B cells in the synovia of JIA patients suggests antigen-driven activation of B cells within the inflamed tissues potentially triggered by local antigens (18). Changes in B cell subpopulations have also been documented in peripheral blood and synovial fluid from JIA patients (12–14, 17, 18, 92, 177–179). Indeed, expansions of CD5+ B cells and transitional ($CD24^{high}CD38^{high}$) B cells have been reported in oJIA and pJIA patients (12–14, 17), which suggest that defects in B cell differentiation and homeostasis may occur in JIA. Of note, CD5+ B cells might be involved in autoantibody production and have the ability to function as antigen presenting cells (180–182). Moreover, it has been demonstrated that transitional B cells ($CD24^{high}CD38^{high}$) can secrete interleukin (IL)-10 and regulate CD4+ T cell proliferation and differentiation toward T helper effector cells (183–186). Furthermore, transitional B cells can also secrete pro-inflammatory cytokines such as IL-6 and tumor necrosis factor (TNF) that contribute to disease pathogenesis (187–189). In addition, alterations in regulatory B cells (Bregs) have also been described in JIA patients that might contribute to disease development (178, 190). Indeed, it was shown that the frequency of $CD19^{+}CD24^{high}CD38^{high}$ Bregs was significantly decreased in peripheral blood and synovial fluid from JIA patients (178). Interestingly, patients with pJIA RF+ had reduced levels of $CD19^{+}CD24^{high}CD38^{high}$ Bregs when compared to patients with pJIA RF- (178). Moreover, the frequency of IL-10-producing Bregs (B10 cells) was significantly lower in active JIA patients in comparison to inactive patients (178). In fact, alterations in regulatory B cell numbers and/ or functions have been described in several autoimmune diseases (183, 191–195). Previous studies have shown that RA patients have decreased frequencies of $CD19^{+}CD24^{high}CD38^{high}$ Bregs in circulation and that these cells fail to suppress Th17 cells differentiation (183). Furthermore, RA patients with active disease have reduced levels of $CD19^{+}CD24^{high}CD38^{high}$ Bregs in peripheral blood when compared to patients with inactive disease, similarly to what has been described in JIA (183). Taken together, these observations suggest that patients with JIA might have altered regulatory B cell functions, with defective suppression of T helper cell subsets (Th1 and Th17) and/ or impaired regulatory T cells activation, as previously described in other autoimmune diseases (**Figure 3C**) (183, 191–195). In addition, increased levels of cytokines relevant for B cell activation, maturation, differentiation and survival such as B cell activating factor (BAFF), A proliferation-inducing ligand (APRIL), IL-6 or IL-21, have been detected in serum and/ or synovial fluid from JIA patients (76, 89, 196–201). Of note, BAFF and APRIL serum levels from JIA patients were significantly correlated with disease activity (199). Furthermore, elevated peripheral blood BAFF mRNA levels have been described in JIA patients (202). Also, increased levels of IL-6 and IL-21, cytokines relevant for B cell maturation and plasma cell differentiation, respectively, have been found in synovial fluid from JIA patients (76). Interestingly, IL-21 synovial fluid concentration was particularly increased in pJIA patients (76). Moreover, it was observed that a worse disease severity at baseline in JIA patients was associated with increased IL-6 plasma levels (201). Overall, these observations support the occurrence of B cell triggering mechanisms in JIA that contribute to disease progression. B cells can also act as major producers of receptor activator of nuclear factor kappa-B ligand (RANKL), a key cytokine in osteoclastogenesis, and bone erosion (167, 169, 203). Interestingly, it has been shown that JIA patients have significantly increased levels of RANKL not only in serum, but also in synovial fluid (99, 204–206). Importantly, higher levels of RANKL were associated with a more serious disease, particularly in pJIA patients (99, 205, 206). Thus, these observations suggest that B cells might have a critical role in bone damage and joint destruction in JIA (**Figure 3D**).

TREATMENT AND B CELL TARGETED THERAPIES IN JUVENILE IDIOPATHIC ARTHRITIS

Treatment of JIA is adjusted according to the severity of the disease as combinations of non-steroidal anti-inflammatory drugs (NSAIDs), synthetic and/ or biological disease modifying anti-rheumatic drugs (DMARDs) (207–210). Intra-articular corticosteroid injections can also be used with great effectiveness (211, 212). Systemic administration of corticosteroids can have a positive short-term effect, but its prolonged administration is

associated with severe side effects such as osteoporosis, growth suppression or immunosuppression (213–215). The American College of Rheumatology (ACR) recommends early use of DMARDs in JIA patients, specifically methotrexate (MTX) (214, 216–218). Furthermore, biological drugs have also been approved for JIA treatment, including TNF inhibitors (etanercept, infliximab, adalimumab, and golimumab) (219–223); the T-cell modulator abatacept (224, 225); the humanized monoclonal antibody against the IL-6 receptor (IL-6R) tocilizumab (226–228) and the IL-1 inhibitor canakinumab (for sJIA) (229–231). Moreover, IL-1R antagonist anakinra (232, 233) and IL-1 inhibitor rilonacept (234, 235) can also be used as effective treatments in sJIA. Additionally, the Janus kinase inhibitor (JAKi) tofacitinib is already approved for the treatment of JIA and clinical trials are currently ongoing to assess the effectiveness and safety of baricitinib in JIA treatment, with preliminary data showing promising results (236–240). Despite the progress achieved in the last years in JIA treatment, about half of the patients continue to require active treatment into adult life, whereas complete remission is reached in only 20–25% of patients (241, 242). B cell depletion therapy with rituximab, a monoclonal antibody that targets CD20 expressed on B cells, is a successful treatment in autoimmune diseases such as RA (243, 244). Nevertheless, few studies have investigated the effectiveness and safety of this treatment option in JIA (7, 245–253). Rituximab is currently only considered in JIA patients refractory to first-line treatments such as TNF inhibitors and standard immunosuppressive therapies, namely MTX (7, 25, 248, 253). Indeed, it has been demonstrated that rituximab is an effective therapeutic option in patients with severe forms of oligoarticular, polyarticular, and systemic JIA, refractory to several prior agents (245–248, 251–253). Adverse events such as infusion reactions, hypogammaglobulinaemia and infections have been reported in pediatric patients after B cell depletion therapy and must be taken into account (254, 255). Nonetheless, rituximab has been reported as an effective and well-tolerated treatment in children, with a low rate of serious infections described in JIA patients, although it is not formally approved by the European Medicines Agency (EMA) for this indication (253). Notably, the efficacy of rituximab treatment in JIA patients strongly supports that B cells play an important role in JIA pathogenesis.

CONCLUSIONS

JIA is the most common rheumatic disorder in children, classified in seven different categories according to ILAR criteria. JIA can cause significant disability and loss of quality of life, if not treated. Disturbances in innate and adaptive immune responses have been implicated in JIA development. B cells are important players in autoimmune diseases and may have roles in JIA pathogenesis through autoantibody production, antigen presentation, cytokine release, and/ or T cell activation. The study of B cells has not been extensively explored in JIA, but evidence from the literature suggests that B cells might have indeed a relevant role in JIA pathophysiology. In fact, the detection of autoantibodies such as ANA, RF and ACPA in JIA patients supports a breakdown in B cell tolerance. Furthermore, altered B cell homeostasis, B cell differentiation, and B cell hyperactivity have been described in JIA, which further supports B cell intervention in disease development. B cell depletion therapy with rituximab is a treatment option considered in JIA patients refractory to first-line biologic treatments such as TNF inhibitors, which has been shown to be an effective and well-tolerated treatment in children with JIA, supporting B cell involvement in JIA pathogenesis. Therefore, further research studies concerning the role of B cells in JIA pathophysiology should be explored, which might be relevant for a better knowledge of disease pathogenesis and have important implications in current and future B-cell targeted therapeutic approaches in JIA.

AUTHOR CONTRIBUTIONS

RAM conceptualized the manuscript, reviewed the literature, and wrote the manuscript. JEF revised the manuscript and contributed with important intellectual input. All authors read and approved the final manuscript.

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Early Changes in B and Plasma Cell Subsets and Traditional Serological Markers as Predictors of SRI-4 Response to Therapy in Systemic Lupus Erythematosus

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Objective: With the premise of the hypothesis that early biological responses to therapy for active systemic lupus erythematosus (SLE) portend later clinical improvements, we studied changes in B cell subsets and traditional serological markers in relation to clinical response to standard therapy (ST) with or without the addition of belimumab.

Patients and Methods: We analyzed data from the BLISS-76, BLISS-SC, and BLISS Northeast Asia trials (N = 1712). Circulating $CD19^+$ B cell subsets were determined by flow-cytometry. We studied associations of relative to baseline percentage changes in circulating B and plasma cell subsets, anti-dsDNA antibody levels and complement levels with SLE Responder Index (SRI)-4 response after 52 weeks of treatment. Changes occurring through week 8 were deemed "rapid," through week 24 "early," and thereafter "delayed".

Results: In the analysis of the entire cohort, SRI-4 responders showed more prominent decreases from baseline through week 52 in $CD19^+CD20^+CD27^-$ naïve B cells (median change: −61.2% versus −50.0%; P = 0.004), $CD19^+CD20^-CD27^{bright}$ plasmablasts (−44.9% versus −33.3%; P = 0.011), and $CD19^+CD20^-CD138^+$ long-lived plasma cells (−48.2% versus −37.1%; P = 0.024), and a more prominent rapid (+92.0% versus +66.7%; P = 0.002) and early (+60.0% versus +49.5%; P = 0.033) expansion of $CD19^+CD20^+CD27^+$ memory B cells than non-responders. More prominent rapid reductions in anti-dsDNA (−14.8% versus −8.7%; P = 0.043) and increases in C3 (+4.9% versus +2.1%; P = 0.014) and C4 levels (+11.5% versus +8.3%; P = 0.017) were documented in SRI-4 responders compared with non-responders among patients who received add-on belimumab, but not among patients who received non-biological ST alone.

Conclusion: SRI-4 responders showed a more prominent rapid expansion of memory B cells and more prominent delayed reductions in naïve B cells, plasmablasts and

long-lived plasma cells. Moreover, clinical response to belimumab was associated with preceding more prominent reductions of anti-dsDNA and increases in C3 and C4 levels. Monitoring biological changes may prove useful in SLE patient surveillance and early treatment evaluation.

Keywords: systemic lupus erythematosus, biomarkers, prediction, B cells, plasma cells, B lymphocyte, belimumab, biologics

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, inflammatory, autoimmune disease that predominantly affects women during their fertile age and is characterized by immense heterogeneity in clinical presentation (1). The treatment of SLE mainly consists of antimalarial agents, glucocorticoids, non-biological disease modifying anti-rheumatic drugs and since recently biological agents (2). The monoclonal antibody belimumab that selectively binds to the soluble counterpart of B cell activating factor (BAFF; also known as B lymphocyte stimulator, BLyS) is licensed for SLE treatment since 2011 (3), and for active lupus nephritis since 2021 (4). The efficacy of belimumab in inducing disease control and reducing the risk of disease flares has been documented in multiple clinical trials and real-life observational studies (5–18), including documentation of its long-term use (19), lending indirect corroboration to the important role of B cells in SLE pathogenesis (1).

Given its mode of action, belimumab is expected to impede the survival and differentiation of B cells, especially in their early stages, as shown in previous research (20–23). Declining counts of B cells could therefore be expected to portend good responses to belimumab therapy, in a similar fashion to successful B cell depletion heralding good clinical responses to rituximab (24, 25). In a real-life observational study of 23 patients with SLE, immunological responses upon commencement of belimumab therapy preceded overt clinical improvements, and low B cell counts were associated with favorable treatment outcome (21). Taken together, we hypothesized that early biological changes upon commencement of belimumab therapy that are consistent with abatement of B cell activity might portend clinical improvements at later timepoints.

Hence, the aim of the present study was to investigate alterations in B and plasma cell subsets as well as selected traditional serological markers in relation to clinical response to therapy for active SLE. More specifically, we investigated B and plasma cell alterations in relation to response to non-biological standard therapy (ST) with or without addition of belimumab, utilizing data from three phase III clinical trials of belimumab in SLE (6–8). Identification of reproducible biological changes that occur soon after treatment commencement and precede clinical response could introduce a novel concept in surveillance of SLE patients, lending promise in early treatment evaluation and thus contributing to a more person-centered therapeutic decision-making and a better use of economic resources.

MATERIALS AND METHODS

Study Population

We designed a *post-hoc* analysis of data from three multicentre, randomized, double-blind, placebo-controlled phase III clinical trials of belimumab i.e., BLISS-76 (NCT00410384) (6), BLISS-SC (NCT01484496) (7), and BLISS Northeast Asia (NEA; NCT01345253) (8). A total of 1712 patients (819, 833, and 60, respectively) were deemed eligible for analysis, based on availability of flow cytometry data for B and plasma cell subsets, along with data on selected serological markers. In these trials, belimumab or placebo was administered intravenously (BLISS-76 and BLISS-NEA; at day 0, 14, and 28 from baseline, and thereafter every 4th week through week 48 in BLISS-NEA and through week 72 in BLISS-76) or subcutaneously (BLISS-SC; belimumab 200 mg or placebo weekly through week 52) on top of non-biological ST, the latter including antimalarial agents, glucocorticoids, immunosuppressants (mainly mycophenolate mofetil, methotrexate, and azathioprine), or combinations thereof.

Briefly, patients were required to have a Safety of Estrogens in Lupus Erythematosus National Assessment - Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) (26) score $\geq$6 (BLISS-76) or $\geq$8 (BLISS-SC and BLISS-NEA) and had to be autoantibody positive, defined as antinuclear antibody titers $\geq$1:80 and/or anti-double stranded (ds)DNA levels $\geq$30 IU/mL. The main exclusion criteria were similar across the three trials and encompassed severe active lupus nephritis or neuropsychiatric SLE, pregnancy, previous treatment with B cell targeting therapy, intravenous cyclophosphamide within 6 months prior to enrollment, and intravenous immunoglobulin, other biologics, prednisone ($>$100 mg/day) or plasmapheresis within 3 months prior to enrollment. All patients had been on stable doses of non-biological ST for at least 30 days prior to belimumab or placebo commencement (baseline). Gradual restrictions regarding allowance in changes in the background immunosuppressive and antimalarial therapy were imposed during the study periods, as well as restrictions regarding glucocorticoid intake. The similar design across the three trials facilitated pooling of data prior to analysis.

Definition of Clinical Response

The primary efficacy endpoint was common across the three trials i.e., the proportion of clinical responders at week 52, with clinical response being defined as attainment of the SLE Responder Index (SRI)-4 criteria (27). SRI-4 response required (i) *a* $\geq$4 point reduction in the SELENA-SLEDAI score compared with baseline

i.e., resolution of at least one SLE disease manifestation, (ii) no new British Isles Lupus Assessment Group (BILAG) (28). A domain score or no more than one new BILAG B score i.e., no significant flares or worsening of the condition, and (iii) no more than a 30% increase in the Physician's Global Assessment (PGA) score (measured on a 0–3 scale) (26), and served as the definition of clinical response in the present analysis.

B Cell Subsets and Serological Markers

Peripheral B and plasma cell subsets were determined with flow cytometry performed within the frame of the BLISS trials (6–8) and subcategorised into total peripheral $CD19^+CD20^+$ B cells, $CD19^+CD20^+CD69^+$ activated B cells, $CD19^+CD20^+CD27^-$ naïve B cells, $CD19^+CD20^+CD27^+$ memory B cells, $CD19^+CD20^-CD27^{bright}$ plasmablasts, $CD19^+CD20^+CD138^+$ short-lived plasma cells, $CD19^+CD20^-CD138^+$ long-lived plasma cells and $CD19^+CD27^{bright}CD38^{bright}$ SLE-associated plasma cells, as previously described (20, 29, 30). Serum levels of anti-dsDNA, C3 and C4 were determined within the frame of the BLISS trials (6–8) and were made available through the Clinical Study Data Request (CSDR) consortium.

We analyzed percentages of relative to baseline (i.e., treatment commencement) changes in B and plasma cell subsets as well as in serum levels of anti-dsDNA, C3, and C4 that occurred through week 8, 24, and 52. Changes occurring through week 8 were deemed rapid, changes occurring through week 24 were deemed early, and changes occurring thereafter were referred to as delayed. We next investigated associations between changes in B cell or plasma cell subsets or changes in serological markers and SRI-4 response at week 52 in the entire patient population, in patients who received add-on belimumab, and in patients who received non-biological ST alone.

Ethics

Data from the BLISS-76, BLISS-SC and BLISS-NEA trials were made available by GlaxoSmithKline (Uxbridge, United Kingdom) through the CSDR consortium. The trial protocols were approved by regional ethics review boards at all participating centers and complied with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all study participants prior to enrollment. The present study was approved by the Swedish Ethical Review Authority (2019-05498).

Statistics

Descriptive statistics are reported as means and standard deviations or medians and interquartile ranges for continuous variables. Frequencies are reported for categorical variables. Values (relative to baseline percentage change) above the 97.5th percentile were treated as extreme values and set to a same max value (equal to the 97.5th percentile) for each cell variable. Comparisons of distributions of relative to baseline changes between groups (SRI-4 responders versus non-responders, and patients who received belimumab versus placebo) were conducted using the non-parametric Mann–Whitney *U* test. *P*-values below 0.05 were deemed statistically significant. All analyses were performed using the R version 4.01 software (R Foundation for Statistical Computing, Vienna, Austria). The GraphPad Prism software version 9 (La Jolla, CA, United States) was used for the preparation of graphs.

RESULTS

Patient Characteristics

Demographics, clinical and serological data of the patients including comparisons between SRI-4 responders and non-responders are reported in **Table 1**. In the pooled dataset, 818/1712 patients (47.8%) attained SRI-4 at week 52. Baseline B and plasma cell data including comparisons between patients who attained and patients who did not attain clinical response at week 52 are reported in **Table 2**, stratified by trial to account for batch variations in flow cytometry readouts across the BLISS trials.

B and Plasma Cell Kinetics in Relation to SRI-4 Response

In the entire patient population i.e., all treatment arms, a more prominent relative to baseline decrease in $CD19^+CD20^+$ B cells was documented among SRI-4 responders compared with non-responders at week 52 (median change: −43.8% versus −34.7%; $P = 0.023$), but not at earlier timepoints (**Figure 1A**). A similar pattern was seen for $CD19^+CD20^+CD27^-$ naïve B cells (−61.2% versus −50.0%; $P = 0.004$; **Figure 1D**), $CD19^+CD20^-CD27^{bright}$ plasmablasts (−44.9% versus −33.3%; $P = 0.011$; **Figure 1G**), and $CD19^+CD20^-CD138^+$ long-lived plasma cells (−48.2% versus −37.1%; $P = 0.024$; **Figure 1F**), as well as in numerical but not statistically significant terms for $CD19^+CD20^+CD69^+$ activated B cells (−43.0% versus −34.4%; $P = 0.300$; **Figure 1C**) and $CD19^+CD27^{bright}CD38^{bright}$ SLE-associated plasma cells (−38.9% versus −28.9%; $P = 0.148$; **Figure 1H**). By contrast, SRI-4 responders were characterized by a more prominent rapid (+92.0% versus +66.7%; $P = 0.002$) and early (+60.0% versus +49.5%; $P = 0.033$) expansion of $CD19^+CD20^+CD27^+$ memory B cells compared with non-responders (**Figure 1B**), with a subsequent return toward baseline values through week 52, resulting in no discrepant change between SRI-4 responders and non-responders (+14.3% versus +16.7%; $P = 0.988$). Results are detailed in the online **Supplementary Material**.

In stratified analysis, differences in relative to baseline B and plasma cell changes between SRI-4 responders and non-responders did not reach statistical significance among patients who received add-on belimumab (any dose or administration route) or among patients who received ST alone (**Figure 1**).

$CD19^+CD20^+$ B cells showed more prominent reductions in patients who received belimumab compared with patients who received ST alone from week 24 onward, with similar patterns observed for $CD19^+CD20^+CD27^-$ naïve B cells, $CD19^+CD20^-CD27^{bright}$ plasmablasts, and $CD19^+CD27^{bright}CD38^{bright}$ SLE-associated plasma cells from week 8 onward. The expanding-returning pattern for $CD19^+CD20^+CD27^+$ memory B cells was only seen in patients who received belimumab, yielding significant differences

TABLE 1 | Characteristics of SRI-4 responders versus non-responders at week 52 in the pooled BLISS study population.

	All patients	SRI-4	No SRI-4	*P*-value
	N = 1712	*N* = 818	*N* = 894	
Patient characteristics				
Age at baseline (years)	39.3 ± 11.9	38.9 ± 11.8	39.6 ± 12.0	0.223
Female sex	1605 (93.8%)	770 (94.1%)	835 (93.4%)	0.532
Ancestry				
Asian	269 (15.7%)	129 (15.8%)	140 (15.7%)	0.950
Black/African American	203 (11.9%)	76 (9.3%)	127 (14.2%)	**0.002**
Indigenous American*	170 (9.9%)	103 (12.6%)	67 (7.5%)	**<0.001**
White/Caucasian	1070 (62.5%)	510 (62.3%)	560 (62.6%)	0.901
Clinical data				
SLE duration at baseline (years)	5.1 (1.6-10.6)	4.6 (1.4-9.6)	5.6 (1.9-11.4)	**0.001**
Treatment at baseline				
Glucocorticoids	1403 (82.0%)	690 (84.4%)	713 (79.8%)	**0.013**
AMA†	1097 (64.1%)	537 (65.6%)	560 (62.6%)	0.195
Immunosuppressants‡	881 (51.5%)	387 (47.3%)	494 (55.3%)	**0.001**
Azathioprine	335 (19.6%)	159 (19.4%)	176 (19.7%)	0.897
Methotrexate	248 (14.5%)	102 (12.5%)	146 (16.3%)	**0.023**
Mycophenolate mofetil or sodium	243 (14.2%)	100 (12.2%)	143 (16.0%)	**0.026**
Trial intervention				
Placebo	575 (33.6%)	232 (28.4%)	343 (38.4%)	**<0.001**
Belimumab	1137 (66.4%)	586 (71.6%)	551 (61.6%)	**<0.001**
i.v. 1 mg/kg	271 (15.8%)	110 (13.4%)	161 (18.0%)	**0.010**
i.v. 10 mg/kg	312 (18.2%)	136 (16.6%)	176 (19.7%)	0.101
s.c. 200 mg	554 (32.4%)	340 (41.6%)	214 (23.9%)	**<0.001**
Serological markers at baseline				
C3; mg/dL	95.5 (74.0-118.0)	96.0 (76.0–118.0)	95.0 (72.0–118.0)	0.298
C4; mg/dL	15.0 (9.0-22.0)	15.0 (9.0–22.0)	14.0 (8.0–22.0)	0.095
Anti-dsDNA; IU/mL (all patients)	95.0 (29.0–287.8)	91.5 (29.0–269.3)	97.5 (29.0–321.5)	0.366
Anti-dsDNA; IU/mL (patients positive at baseline)	167.0 (89.0–495.8); *N* = 1170	149.0 (82.8–438.5); *N* = 570	189.5 (97.0–527.5); *N* = 600	**0.023**

*Data are presented as number (percentage), mean ± standard deviation, or median (interquartile range), as appropriate. In case of missing values, the total number of patients with available data is indicated. Statistically significant P-values are in bold. *Alaska Native or American Indian from North, South, or Central America. †Hydroxychloroquine, chloroquine, mepacrine, mepacrine hydrochloride or quinine sulfate. ‡Azathioprine, cyclosporine, oral cyclophosphamide, leflunomide, methotrexate, mizoribine, mycophenolate mofetil, mycophenolate sodium, or thalidomide. AMA, antimalarial agents; C3, complement component 3; C4, complement component 4; i.v., intravenous; s.c., subcutaneous; SLE, systemic lupus erythematosus; SRI-4, SLE Responder Index 4.*

compared with patients who received ST alone, yet irrespective of SRI-4 response (**Figure 1** and **Supplementary Material**).

Changes in Serological Markers in Relation to SRI-4 Response

In the entire patient population, a more prominent relative to baseline decline in anti-dsDNA levels was documented in SRI-4 responders compared with non-responders as early as 8 weeks after therapy commencement (−8.3% versus 0.0%; $P = 0.006$). This difference persisted for changes in anti-dsDNA levels from baseline through week 24 (−21.8% versus 0.0%; $P < 0.001$) and week 52 (−34.8% versus −2.0%; $P < 0.001$; **Figure 2A**), and was also seen in the subgroup of patients with baseline anti-dsDNA levels above the threshold for positivity (≥30 IU/mL) from baseline through week 8 (−20.6% versus −16.7%; $P = 0.005$), week 24 (−34.8% versus −20.3%; $P < 0.001$) and week 52 (−48.7% versus −28.3%; $P < 0.001$; **Figure 2B**). Similarly, a more prominent increase in C3 levels was seen in SRI-4 responders compared with non-responders from baseline through week 8 (+3.3% versus +1.0%; $P = 0.012$) and week 52 (+6.3% versus 0.0%; $P < 0.001$; **Figure 2C**), as well as in C4 levels from baseline through week 8 (+8.5% versus +5.4%; $P = 0.003$), week 24 (+12.5% versus +10.0%; $P = 0.017$) and week 52 (+18.2% versus +10.0%; $P < 0.001$; **Figure 2D**).

In stratified analysis, differences in relative to baseline reductions in anti-dsDNA levels and increases in C3 and C4 levels were overall more prominent in patients who received add-on belimumab than in patients who received ST alone (**Supplementary Material**). Notably, during the rapid phase i.e., from baseline through week 8, we observed more prominent reductions in anti-dsDNA levels in patients who attained SRI-4 response compared with non-responders within the belimumab-treated population (−14.8% versus −8.7%; $P = 0.043$; **Figure 2A**), but not within patients who received ST alone. In a similar fashion, the rapid increases in C3 and C4 levels were more

TABLE 2 | B cell subset counts at baseline in SRI-4 responders versus non-responders at week 52 in the BLISS-76, BLISS-SC, and BLISS Northeast Asia study population.

B cell subsets	All patients	SRI-4	No SRI-4	*P*-value
BLISS-76				
	***N* = 819**	***N* = 320**	***N* = 499**	
$CD19^+CD20^+$ ($x10^3$/mL)	91.5 (43.0–176.0); *N* = 756	97.0 (42.3–187.0); *N* = 292	88.0 (43.3–166.8); *N* = 464	0.306
$CD19^+CD20^+CD27^+$ ($x10^3$/mL)	14.0 (6.0–27.0); *N* = 756	15.0 (7.0–27.0); *N* = 292	14.0 (6.0–26.0); *N* = 464	0.191
$CD19^+CD20^+CD69^+$ (/mL)	2096.5 (938.3–4350.8); *N* = 744	2230.0 (721.0–4408.0); *N* = 287	2071.0 (1017.0–4322.0); *N* = 457	0.631
$CD19^+CD20^+CD27^-$ ($x10^3$/mL)	75.0 (33.0–143.0); *N* = 756	81.0 (32.0–151.8); *N* = 292	72.0 (34.0–134.3); *N* = 464	0.377
$CD19^+CD20^+CD138^+$ (/mL)	819.0 (334.0–1811.5); *N* = 749	832.0 (315.0–1772.0); *N* = 289	802.5 (345.3–1820.0); *N* = 460	0.654
$CD19^+CD20^-CD138^+$ (/mL)	482.5 (211.0–1067.3); *N* = 748	483.5 (199.0–1028.0); *N* = 288	481.0 (220.0–1098.0); *N* = 460	0.499
$CD19^+CD20^-CD27^{brt}$ (/mL)	299.0 (115.0–705.0); *N* = 747	350.0 (115.0–713.0); *N* = 287	282.0 (115.5–685.0); *N* = 460	0.224
$CD19^+CD27^{brt}CD38^{brt}$ (/mL)	306.0 (116.0–701.8); *N* = 754	315.0 (121.0–760.0); *N* = 291	301.0 (113.0–677.0); *N* = 463	0.296
BLISS–SC				
	***N* = 833**	***N* = 475**	***N* = 358**	
$CD19^+CD20^+$ ($x10^3$/mL)	106.5 (56.0–196.0); *N* = 808	106.5 (59.0–198.5); *N* = 462	106.5 (53.0–193.3); *N* = 346	0.589
$CD19^+CD20^+CD27^+$ ($x10^3$/mL)	14.0 (7.0–29.0); *N* = 808	14.0 (7.0–30.0); *N* = 462	14.0 (7.0–25.0); *N* = 346	0.320
$CD19^+CD20^+CD69^+$ (/mL)	80.0 (33.0–200.5); *N* = 808	87.5 (34.0–216.0); *N* = 462	74.5 (31.0–176.3); *N* = 346	0.131
$CD19^+CD20^+CD27^-$ ($x10^3$/mL)	89.0 (43.0–167.0); *N* = 808	88.0 (45.8–168.5); *N* = 462	91.0 (41.8–166.0); *N* = 346	0.756
$CD19^+CD20^+CD138^+$ (/mL)	53.0 (20.0–126.8); *N* = 808	52.5 (20.0–131.3); *N* = 462	55.0 (19.0–126.3); *N* = 346	0.925
$CD19^+CD20^-CD138^+$ (/mL)	202.0 (67.3–504.8); *N* = 808	194.5 (67.0–504.3); *N* = 462	212.0 (70.5–508.3); *N* = 346	0.498
$CD19^+CD20^-CD27^{brt}$ (/mL)	2000.0 (1000.0–4000.0); *N* = 808	2000.0 (1000.0–4000.0); *N* = 462	2000.0 (1000.0–4000.0); *N* = 346	0.158
$CD19^+CD27^{brt}CD38^{brt}$ (/mL)	1732.5 (738.0–3933.5); *N* = 808	1802.5 (753.8–3979.3); *N* = 462	1620.5 (705.8–3905.3); *N* = 346	0.252
BLISS Northeast Asia				
	***N* = 60**	***N* = 23**	***N* = 37**	
$CD19^+CD20^+$ ($x10^3$/mL)	52.5 (22.8–96.8); *N* = 54	49.0 (28.0–89.0); *N* = 21	54.0 (21.5–108.0); *N* = 33	0.852
$CD19^+CD20^+CD27^+$ ($x10^3$/mL)	7.3 (3.7–10.6); *N* = 55	7.5 (4.2–13.2); *N* = 21	7.0 (3.6–10.6); *N* = 34	0.716
$CD19^+CD20^+CD69^+$ (/mL)	101.3 (45.9–183.0); *N* = 55	115.8 (51.9–228.8); *N* = 21	91.3 (45.2–180.0); *N* = 34	0.533
$CD19^+CD20^+CD27^-$ ($x10^3$/mL)	39.7 (18.6–87.5); *N* = 55	39.4 (25.3–82.4); *N* = 21	43.1 (17.3–90.6); *N* = 34	0.986
$CD19^+CD20^+CD138^+$ (/mL)	108.2 (58.1–258.1); *N* = 55	82.4 (57.5–169.6); *N* = 21	114.1 (54.9–377.6); *N* = 34	0.446
$CD19^+CD20^-CD138^+$ (/mL)	303.1 (174.5 –668.8); *N* = 55	233.8 (137.3–604.2); *N* = 21	340.2 (196.2–721.0); *N* = 34	0.188
$CD19^+CD20^-CD27^{brt}$ (/mL)	916.5 (262.8–2008.4); *N* = 55	696.7 (195.8–1319.2); *N* = 21	1037.0 (345.5–2951.4); *N* = 34	0.188
$CD19^+CD27^{brt}CD38^{brt}$ (/mL)	934.9 (264.7–2095.6); *N* = 55	741.8 (210.8–1451.0); *N* = 21	985.6 (405.0–2522.4); *N* = 34	0.260

Data are presented as medians (interquartile range) of absolute counts. In case of missing values, the total number of patients with available data is indicated. P-values are derived from non-parametrical Mann–Whitney U tests. Statistically significant P-values are in bold. SC, subcutaneous; SRI-4, Systemic Lupus Erythematosus Responder Index 4.

prominent in SRI-4 responders compared with non-responders in belimumab-treated patients (+4.9% versus +2.1%; $P = 0.014$; **Figure 2C** and +11.5% versus +8.3%; $P = 0.017$; **Figure 2D**, respectively), but not in patients who received ST alone.

DISCUSSION

We investigated alterations across different circulating B and plasma cell subsets as well as selected traditional serological markers upon treatment for active SLE and their associations with clinical response documented 52 weeks after treatment commencement. We demonstrated that $CD19^+CD20^+$ B cells decreased more prominently in responders than in non-responders to therapy, particularly $CD19^+CD20^+CD27^-$ naïve B cells. Moreover, $CD19^+CD20^-CD27^{bright}$ plasmablasts and $CD19^+CD20^-CD138^+$ long-lived plasma cells also decreased more prominently in clinical responders than in non-responders. However, this separation for both B cell and plasma cell subsets became significant only for the delayed follow-up phase i.e., for relative to baseline changes through week 52. By contrast, clinical responders showed a more prominent rapid expansion of $CD19^+CD20^+CD27^+$ memory B cells compared with non-responders. While memory B cells tended to return toward baseline values thereafter, this separation between responders and non-responders was also present in the early phase i.e., from baseline through week 24. After stratification into active arm and placebo, it became evident that this expanding-returning pattern for memory B cells was induced by belimumab, a phenomenon that has been highlighted in previous studies (20, 21, 23).

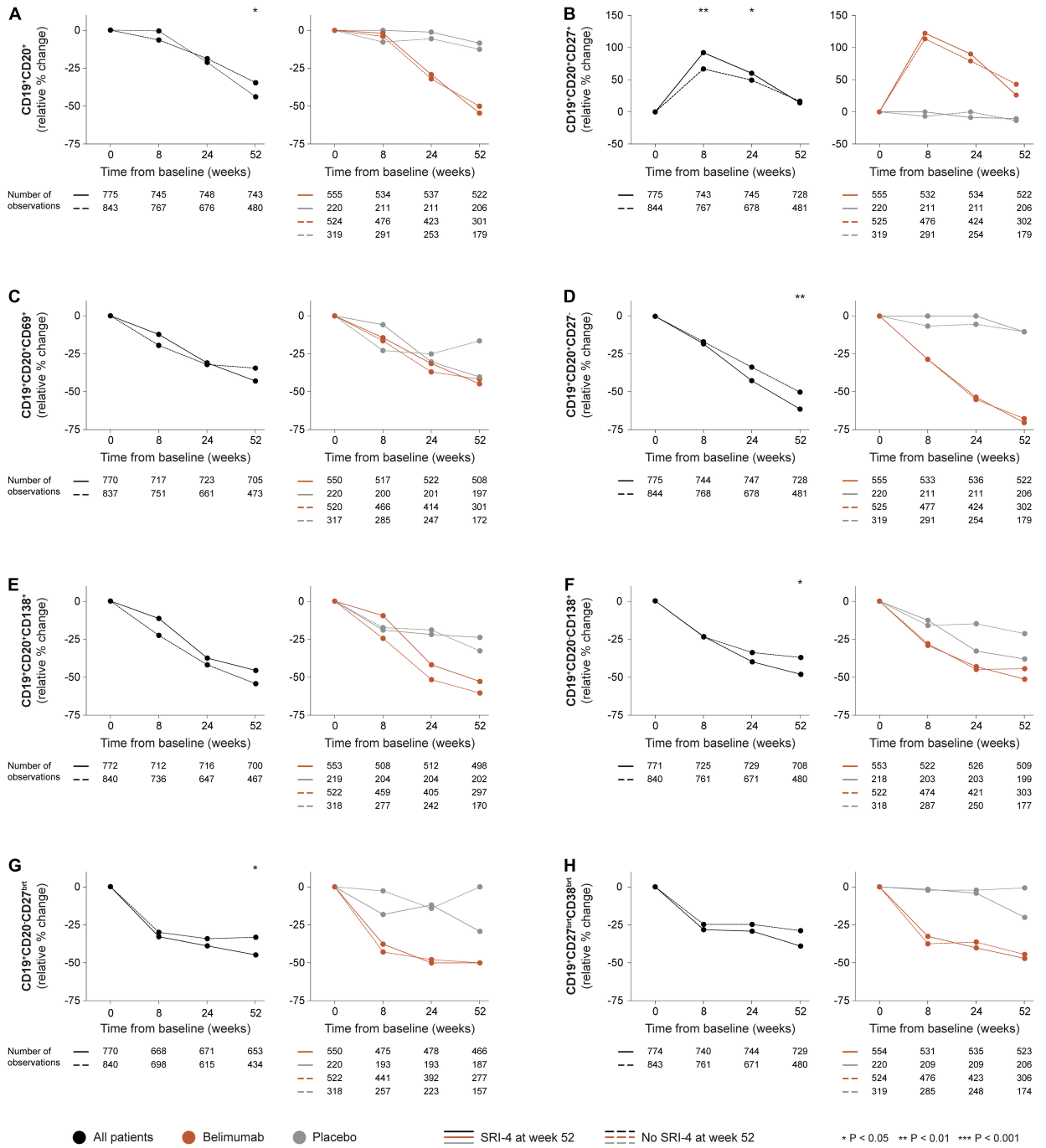

FIGURE 1 | B and plasma cell subset alterations in relation to clinical response. The graphs delineate relative to baseline percentage changes in selected B cell and plasma cell subsets in patients who attained SRI-4 response at week 52 from baseline (continuous lines) and patients who did not (dashed lines). Comparisons between SRI-4 responders and non-responders were conducted for the entire population with available data (black lines), and after stratification into patients who received standard therapy plus belimumab (terracotta lines) and patients who received standard therapy alone (gray lines). *P*-values derived from non-parametric Mann–Whitney *U* tests. The number of patients with available data at each timepoint is indicated for each patient subgroup. SRI, Systemic lupus erythematosus Responder Index.

Furthermore, we demonstrated that reductions in anti-dsDNA and increases in C3 and C4 levels distinguished clinical responders from non-responders during follow-up, with significant separations documented as early as 8 weeks after treatment initiation. After stratification into active arm and placebo, this separation remained significant for both

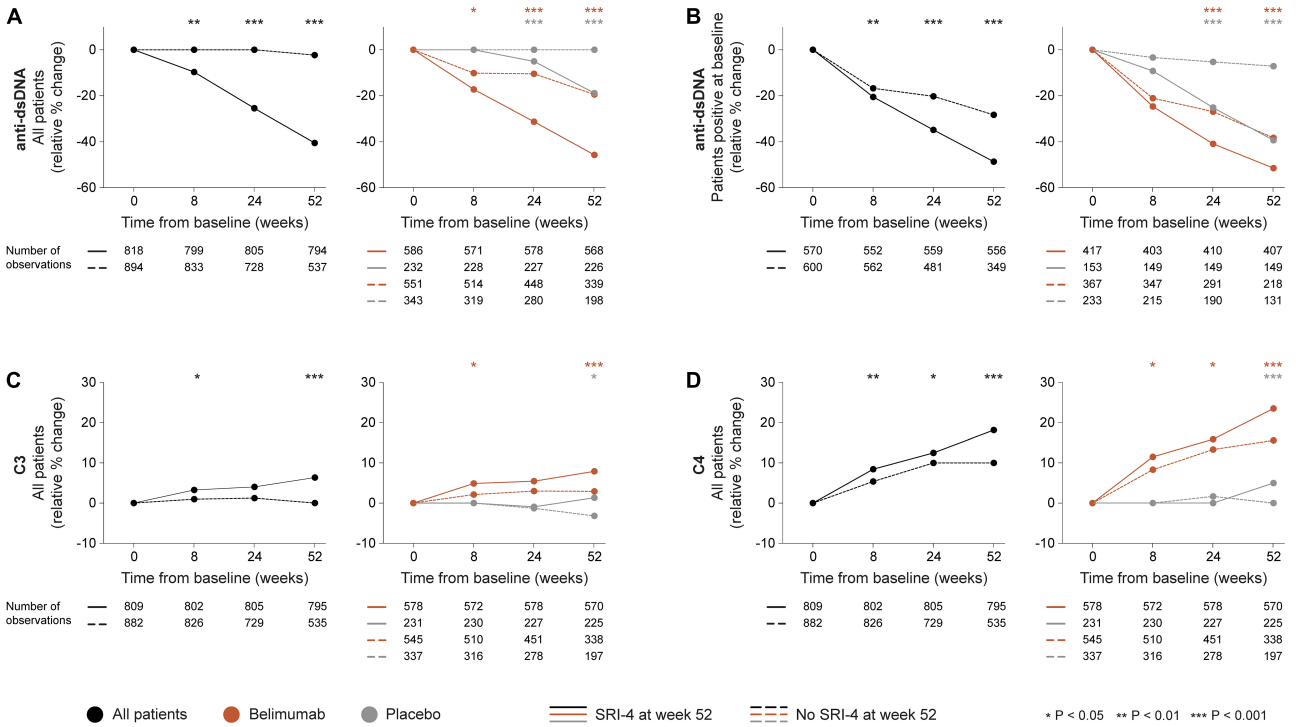

FIGURE 2 | Changes in selected serological markers in relation to clinical response. The graphs delineate relative to baseline percentage changes in anti-dsDNA, C3, and C4 levels in patients who attained SRI-4 response at week 52 from baseline (continuous lines) and patients who did not (dashed lines). Comparisons between SRI-4 responders and non-responders were conducted for the entire population with available data (black lines), and after stratification into patients who received standard therapy plus belimumab (terracotta lines) and patients who received standard therapy alone (gray lines). For anti-dsDNA levels, a separate analysis for patients with positive anti-dsDNA levels (≥30 IU/mL) at baseline is also demonstrated. *P*-values derived from non-parametric Mann–Whitney *U* tests. The number of patients with available data at each time point is indicated for each patient subgroup. Anti-dsDNA: anti-double stranded DNA antibodies; C3: complement component 3; C4: complement component 4; SRI: Systemic lupus erythematosus Responder Index.

belimumab-treated patients and patients who received ST alone at the evaluation of the delayed phase, but was only present in belimumab-treated patients during the rapid phase, suggesting that belimumab induces rapid and sustained changes in these serological markers, which are more prominent in patients who will show clinical response to treatment and could thus serve as useful markers in early treatment evaluation.

Several of these findings warrant further discussion. In the first place, belimumab was shown to induce rapid and sustained decreases in plasma cell subsets, with a clear separation from the placebo group irrespective of response to treatment. This finding is of interest in light of previous literature that has shown rather delayed or no plasma cell affection by belimumab therapy (21–23). This discrepancy may at least partly be due to the large SLE population in the present study which amplified the power in statistical calculations, and to some extent due to the detailed characterization of plasma cells into different subsets.

Another point of interest was the expanding-returning pattern of memory B cells, which herein showed ability to separate between clinical responders and non-responders. This is in line with our previous findings that a rapid expansion of memory B cells is associated with a lower probability of severe flare and renal flare (31). After stratification into the belimumab and placebo arms, this expanding-returning pattern of memory B cells was only seen in patients treated with add-on belimumab, illustrating a phenomenon induced by belimumab that has been documented in several previous studies (20, 21, 23). While preservation of memory B cells upon belimumab therapy may be hypothesized to be due to the fact that their survival is not dependent on BAFF (32), the explanation underlying the rapid expansion and subsequent return of memory B cells has not been thoroughly elucidated. In fact, serum levels of a proliferation inducing ligand (APRIL), the tumour necrosis factor (TNF) ligand superfamily member that is most homologous to BAFF (33, 34), have been shown to decrease during belimumab therapy (13), suggesting that APRIL is to a larger extent consumed on its receptors on the surface of B cells in the milieu of a dearth of biologically active BAFF. Based on the known effects of BAFF on B cells (35) as well as early proof-of-concept studies on animals (36) and a phase II trial of belimumab (37), Stohl et al. speculated that the expanding-returning pattern of memory B cells may be a result of release from disrupted germinal centers where memory B cells reside, or a result of inhibition of their return to these lymphoid tissues, or a consequence of enhanced B cell differentiation from naïve to memory B cells (20). Findings from a recent study by Arends et al. suggested that this phenomenon may be

due to secondarily disrupted lymphocyte trafficking owing to downregulated expression of genes coding for migration markers such as L-selectin (also known as CD62L) and intercellular adhesion molecule 2 (ICAM2; also known as CD102), which might prevent homing of lymphocytes to inflamed tissues and culminate in an abundance of memory B cells in the bloodstream (38). Collectively, this pattern of memory B cells during the rapid and early phases of belimumab therapy may not only have interest in terms of underlying biology, but also in terms of usefulness in the early evaluation of belimumab therapy where a lack of this pattern may signify lower probability of clinical response.

Another interesting finding was the ability of anti-dsDNA and complement level kinetics to separate between responders and non-responders as early as 8 weeks from treatment commencement, with a continuous and even more prominent separation during later timepoints. Importantly, while a delayed separation was present irrespective of the therapeutic regimen, rapid reductions of anti-dsDNA levels and rapid increases of C3 and C4 levels were more prominent in responders than in non-responders among belimumab-treated patients. These findings are in line with previous reports of biological changes preceding the overt clinical improvement induced by belimumab (21), and while changes in these serological markers might theoretically be expected to follow the kinetics of B cells, the demonstration that these traditional serological markers were more sensitive to change than B cell subsets and preceded B cell reductions and clinical response illustrates that their interrelationship is not always consequential. In this regard, it should also be noted that the choice of SRI-4 for the determination of clinical response may have magnified the impact of anti-dsDNA and complement level kinetics over B cell alterations since dsDNA binding and complement consumption are integral items of the SELENA-SLEDAI, one of the components of SRI-4, which is not the case for B cells.

It is important to clarify that monitoring early biological changes to portend therapeutic outcome should not be considered contradicting to baseline predictors, but complemental toward optimized person-centered surveillance. In fact, while serological status at baseline has been shown to predict the outcome of belimumab therapy in some studies (39, 40), this has not been consistent throughout the literature (13, 15, 41, 42). In a recent study that investigated selected autoantibodies and cytokines as predictors of response to belimumab therapy, early decreases in serum levels of interleukin (IL)-6 showed merit (43). Along the same lines, our study introduces the concept of rapid and early kinetics of selected markers, herein anti-dsDNA and complement levels in particular, as a complemental surveillance tool that may prove useful in early treatment evaluation.

This study has some limitations. Firstly, it was a *post-hoc* analysis of trials which were not designed to address the research question of the present study, which may have hampered the power in stratified statistical analyses. Secondly, the study participants comprised a selected SLE population with primarily musculoskeletal and mucocutaneous activity at baseline and excluded patients with severe active lupus nephritis and severe active central nervous system disease, which limits the generalizability of the findings to real-life SLE populations. Lastly, the characterization of B cell subsets and measurement of serological markers within the frame of three different trials may have introduced confounding due to batch effects, which limited us from studying absolute changes and necessitated investigation of changes relative to baseline. Nevertheless, the study encompassed a large number of patients that commenced therapy for active autoantibody positive SLE and were followed up in a structured manner within the frame of controlled phase III clinical trial programmes, ensuring diligent data collection and scarce occurrence of data missingness.

In summary, we demonstrated that SRI-4 responders showed a more prominent rapid expansion of memory B cells and more prominent delayed reductions in naïve B cells, plasmablasts and long-lived plasma cells. Moreover, clinical response established 1 year after commencement of belimumab therapy was preceded by more prominent rapid reductions of anti-dsDNA and more prominent rapid increases in C3 and C4 levels than in patients who did not respond to therapy. Our findings lend support for the usefulness of B and plasma cell kinetics as a complement to clinical features and traditional serological markers in treatment evaluation, and suggest that surveillance of anti-dsDNA and complement level kinetics may prove helpful in early evaluation of belimumab therapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Swedish Ethical Review Authority. The patients/participants provided their written informed consent to participate in the BLISS trials.

AUTHOR CONTRIBUTIONS

IP and MG: study conception and design. IP, AG, JL, and JC: acquisition of data. IP, AG, JL, AD, and MG: analysis and interpretation of data. All authors were involved in the drafting of the manuscript or revising it critically for important intellectual content and approved the final version to be submitted for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.852162/full#supplementary-material

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