EMERGING ROLES FOR TYPE 2-ASSOCIATED CELLS AND CYTOKINES IN CANCER IMMUNITY

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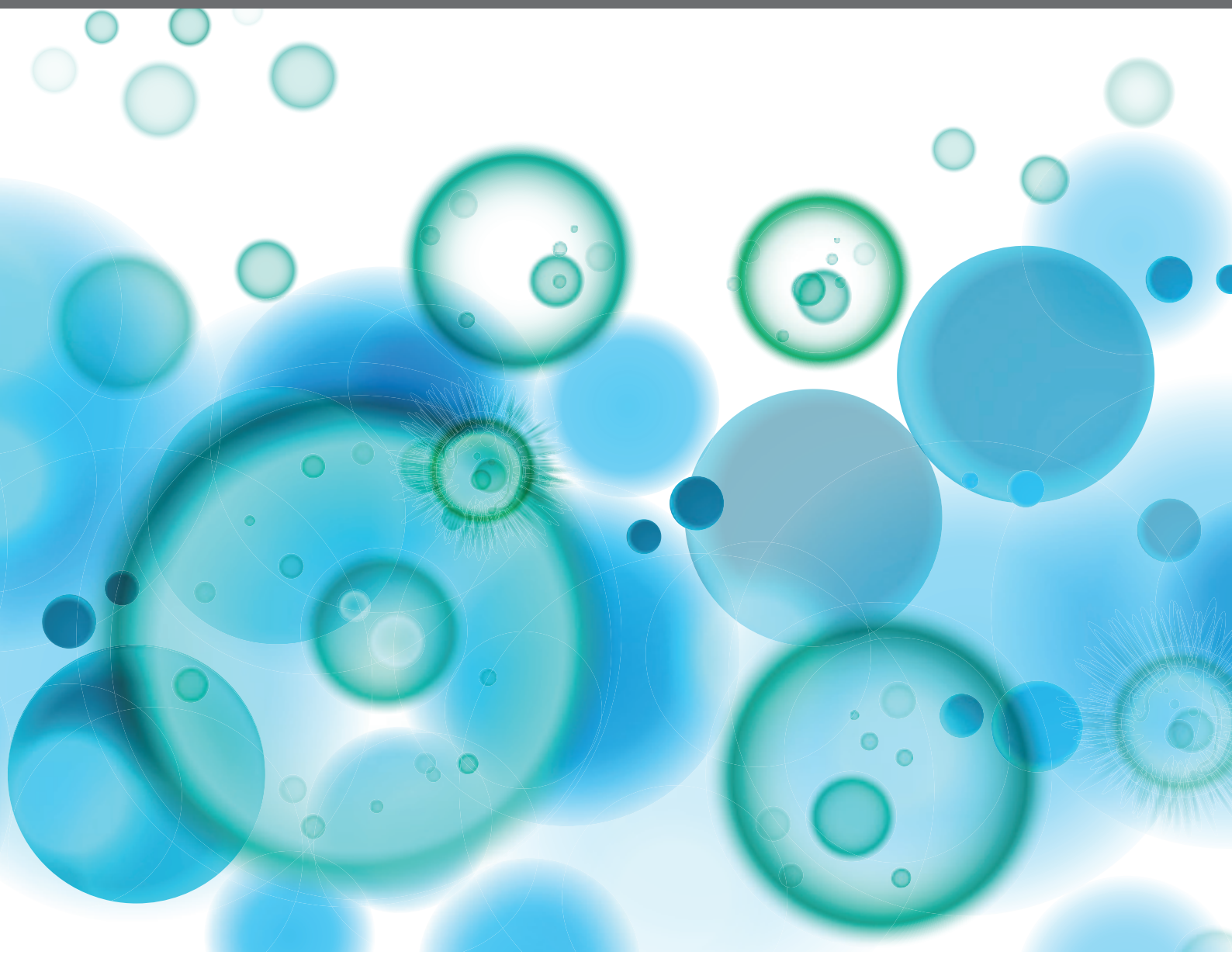

frontiers Research Topics

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EMERGING ROLES FOR TYPE 2-ASSOCIATED CELLS AND CYTOKINES IN CANCER IMMUNITY

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EDITORIAL
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Editorial: Emerging Roles for Type 2-Associated Cells and Cytokines in Cancer Immunity

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Editorial on the Research Topic

Emerging Roles for Type 2-associated Cells and Cytokines in Cancer Immunity

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Type 2 immune responses have mainly been studied in the context of parasite infections and allergic diseases. However, emerging evidence suggests important roles for Type 2 immunity in multiple additional physiological and pathological settings (1). In fact, Type 2-associated cells, such as eosinophils, mast cells, basophils, Type 2 innate lymphoid cells (ILC2), as well as the cytokines IL-4, IL-5, IL-13, IL-33 and thymic stromal lymphopoietin (TSLP) are now considered a significant signaling and effector cell axis in metabolism, tissue remodeling, neuroinflammation and cancer. This Research Topic collects relevant studies on the emerging role of some Type 2-associated cells and cytokines in the modulation of cancer progression and anti-tumor immune responses.

Among the Type 2 cytokines, IL-33 and TSLP are epithelial-derived alarmins capable of activating a plethora of innate and adaptive immune cell populations, thereby affecting tumor immune control. Some Type 2-associated cells, such as mast cells, eosinophils and basophils, display pleiotropic activities within the tumor microenvironment (TME). On the one hand, they can provide growth and angiogenic factors to promote tumor growth. On the other hand, they can exert tumor cytotoxicity through degranulation and release of soluble mediators. In a mini review article, Eissmann et al. debate the role of the IL-33/ST2 axis and mast cells in the regulation of gastrointestinal cancer. Mast cells can regulate IL-33 bioactivity through the release of tryptase and chymase that cleave the immature full-length form of IL-33 into the mature form of the cytokine. Several immune cells, including mast cells, express ST2 at various levels on their cells surface and respond to IL-33 in the TME of gastrointestinal cancers. During pre-cancerous inflammation, a functional cooperation between IL-33 and mast cells promotes the restoration of the epithelial barrier and the regeneration of gastrointestinal epithelium. In colorectal cancers however, albeit the expression of IL-33 and the prevalence of mast cells are not necessarily associated, both appear to have a tumor-promoting role in most pre-clinical and human studies. In gastric cancers, the authors discuss an interesting study involving an IL-33/mast cell/macrophage axis that promotes tumor growth. Here, activation of mast cells by IL-33 resulted in the secretion of macrophage attracting factors and subsequent accumulation of pro-angiogenic and pro-tumorigenic macrophages in the gastric tumors. Finally, the authors discuss the possibility of

harnessing the IL-33/mast cell axis taking into account the dichotomous role they play in gastrointestinal tumors and other cancers.

IL-33 is a pleiotropic cytokine that can activate both innate and adaptive immune cells (and stimulate both Th1 and Th2 responses), which result in dichotomous roles in tumor immunity, clearly depending on the tumor type and the nature of the TME. In another mini review article, Andreone et al. focus on the anti-tumoral mechanisms exerted by IL-33, *via* stimulation of several different immune effector cells. Recent literature indicates that IL-33 can sustain the effector activity of $CD8^+$ T cells and NK cells and promote the functions of $CD4^+$ T lymphocytes in the TME. Moreover, IL-33 can activate the cytotoxic functions of eosinophils and basophils promoting tumor killing. In addition, IL-33/ST2 stimulation enhances the anti-tumor functions of ILC2 cells through multiple mechanisms, including recruitment of eosinophils and cross-presenting DCs, ultimately resulting in tumor cytotoxicity. Finally, recent evidence demonstrating that IL-33 can modulate the expression of immune checkpoints (i.e., PD-1/PD-L1 and CTLA-4) in certain immune cells opens perspectives for targeting the IL-33/ST2 axis to increase immunotherapy efficacy. This is of specific interest as although immune checkpoint inhibitors are one of the most promising avenues in cancer immunotherapy only a subset of patients demonstrate a clinical response. Therefore, it is critically important to identify predictive biomarkers for a beneficial response to ICI therapy and to understand the role of all immune cells and soluble mediators in cancer immunotherapy.

Another epithelial-derived alarmin, TSLP, is a lymphopoietin commonly expressed in the skin, gut and lung tissues. Expression of TSLP and/or its receptor is found in several human cancers and may be associated with the induction of a Type 2-prone TME. In a review article, Protti and De Monte debate Th2-dependent and Th2-independent roles of TSLP in several cancer types and the possible therapeutic targeting of this cytokine. Several studies on human and mouse tumors found either pro- or anti-tumor activity of TSLP, mostly based on the association between TSLP expression and the development of predominant Th2 inflammation in the tumor, or direct TSLP signaling on tumor cells. Protti and De Monte propose a model by which TSLP released by tumor cells and cancer-associated fibroblasts can activate Type 2 immunity to foster cancer progression *via* myeloid TSLP-expressing DCs and M2 macrophages in the stroma, which ultimately allow TSLP-DCs to activate Th2 pro-tumoral responses in the tissue draining lymph node. In view of the pro-tumor *vs*. antitumor function of TSLP in different tumor types, its possible manipulation for therapeutic purposes will need further investigation.

Finally, in a detailed review, Marone et al. discuss the functional characteristics of mouse and human basophils, and how these may dictate tumor fate. Basophils are a Type 2-associated cell subset whose role in cancer is only beginning to be studied. They are a rare immune cell subset representing <1% of human peripheral blood leukocytes but they can accumulate in inflamed tissues and possess powerful effector mechanisms. Basophils can modulate cancer progression through the production of a plethora of angiogenic factors (e.g., VEGF-A, VEGF-B, HGF, ANGPT1 and CXCL8). Furthermore, basophils can release DNA extracellular traps (ETs), which have an antibacterial function but may also promote metastasis and cancer-associated thrombosis, as described for neutrophil ETs. Basophils can also produce a variety of cytokines (e.g., IL-3, IL-4, IL-6 and IL-13) and display plasticity of phenotype and function under the influence of the TME. In addition, under appropriate stimulation, basophils can acquire tumoricidal properties *in vitro*. The generation of genetically engineered mouse models has allowed studying the functional role of basophils in pancreatic ductal adenocarcinoma (PDAC) *in vivo*. Here, the authors propose a model in which basophils recruited to the tumor-draining lymph nodes (TDLN) skew towards Th2 and M2 polarization through the production of IL-4 and by this mechanism play a relevant pro-tumorigenic role in PDAC progression. Overall, this review underlines that despite the established presence of basophils in human and experimental cancers, further investigation is required to elucidate the role of basophils in tumor immunity.

In sum, it is becoming increasingly clear that Type 2-associated cells and soluble mediators are an important part of the TME and can be central orchestrators of cancer development. The published articles in this Research Topic aim to provide a better understanding of the mechanisms operated by Type 2 immune effectors and their interplay with other components of the TME in specific tumor types. We hope this collection will inspire future work exploring and harnessing these overlooked immune components for future therapeutic strategies against cancer.

AUTHOR CONTRIBUTIONS

GS wrote the editorial and invited authors to contribute the Research Topic. AM co-wrote the editorial and invited authors to contribute to the Research Topic. JS co-wrote the editorial and invited authors to contribute to the Research Topic. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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IL33 and Mast Cells—The Key Regulators of Immune Responses in Gastrointestinal Cancers?

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The Interleukin (IL-)1 family IL33 is best known for eliciting type 2 immune responses by stimulating mast cells (MCs), regulatory T-cells (Tregs), innate lymphoid cells (ILCs) and other immune cells. MCs and IL33 provide critical control of immunological and epithelial homeostasis in the gastrointestinal (GI) tract. Meanwhile, the role of MCs in solid malignancies appears tissue-specific with both pro and anti-tumorigenic activities. Likewise, IL33 signaling significantly shapes immune responses in the tumor microenvironment, but these effects remain often dichotomous when assessed in experimental models of cancer. Thus, the balance between tumor suppressing and tumor promoting activities of IL33 are highly context dependent, and most likely dictated by the mixture of cell types responding to IL33. Adding to this complexity is the promiscuous nature by which MCs respond to cytokines other than IL33 and release chemotactic factors that recruit immune cells into the tumor microenvironment. In this review, we integrate the outcomes of recent studies on the role of MCs and IL33 in cancer with our own observations in the GI tract. We propose a working model where the most abundant IL33 responsive immune cell type is likely to dictate an overall tumor-supporting or tumor suppressing outcome *in vivo*. We discuss how these opposing responses affect the therapeutic potential of targeting MC and IL33, and highlight the caveats and challenges facing our ability to effectively harness MCs and IL33 biology for anti-cancer immunotherapy.

Keywords: interleukin 33 (IL33), mast cell (MC), innate immunity, ST2, gastrointestinal (GI) malignancies, tumor microenvironment (TME), therapy targets, cytokine signaling

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INTRODUCTION

The tumor microenvironment (TME) is a complex collection of cellular and extra cellular matrix (ECM) components. Interactions and communications between the various components of the TME are orchestrated by a multitude of signaling molecules, including the cytokine interleukin (IL)33. IL33 was first discovered in 2003 as a nuclear factor in HEVEC cells (NF-HEV) (1) and later identified as an IL1 family cytokine and ligand for the interleukin 1 receptor like 1 receptor (IL1RL1, or commonly referred to as ST2) (2).

IL33 is expressed in fibroblasts, endothelial and epithelial cells (1, 3, 4) as well as in many cancer cells [reviewed in (5, 6)]. Depending on stimulation or disease context, this cytokine is produced by additional cells such as MCs (7), dendritic cells, macrophages, neutrophils, eosinophils, B cells and red blood cells (8–11). Anatomically, the expression of IL33 is highest in barrier tissues like the skin, the air ways and the GI tract, where IL33 release activates innate and adaptive immune

responses upon tissue injury or various infections [reviewed in (12)]. Indeed, tissue resident innate immune cells are the proposed first responder for released IL33, and MCs are present at all these environment-tissue interfaces (13). In general, necrotic or necroptotic cell death is required for its release (14–21), nevertheless, multiple studies suggest release of IL33 from living cells (22–25), suggesting various modes of active secretion and passive release with and without necrotic/necroptotic cell death depending on cell type and stimuli. Further research is required to unravel the exact mechanisms of IL33 release.

IL33 cytokine exerts its activity via binding to a heterodimeric receptor consisting of its primary receptor ST2 and a co-receptor, IL1 receptor accessory protein (IL1RAP) (26, 27) triggering downstream signaling pathways including MYD88, IRAK1/4, MAP kinases and NF-kB (2, 12). Importantly, the various biological outputs following engagement of the IL33-ST2 axis are heavily dictated by the cellular context, which we will further summarize in this review, with a special focus on interaction and importance the innate-immune mast cells for IL33 signaling in cancer. Besides acting as an extracellular ligand conferring activity through its cognate ST2 receptor on targets cells, ST2-independent nuclear IL33 can act as transcriptional repressor in fibroblast, endothelial and immune cells (28, 29). Likewise, nuclear IL33 also promotes immune suppressive functions independent of ST2 in regulatory T (Tregs) cells (30), and cell intrinsic IL33 plays a role in B cell development (31).

IL33—RESPONSIVE CELLS IN THE TUMOR MICROENVIRONMENT

Since the identification of ST2 as the cognate receptor of IL33, various cell types have been shown to express ST2 and to respond to IL33 stimulation. However, there is a significant difference in the quality and quantity of ST2 expression among various cell types. Innate lymphoid cell type 2 (ILC2), Tregs and MCs express the ST2-receptor constitutively, while all other cell types that respond to extracellular IL33 are either ST2 negative at steady-state and only induce ST2 expression upon activation, or express ST2 on minor cell subsets in specific biological processes in a tissue-dependent manner (32).

ILC2 Cells

A significant subset of innate lymphoid cell type 2 (ILC2) are constitutive ST2 expressers. However, the proportion of ST2 positive ILC2s can vary depending on tissue origin and disease context (32–37). Stimulation of ILC2s by IL33/ST2-signaling is critical for their activation, induces their expansion within tissues and triggers secretion of the type 2 cytokines IL-5 and IL-13. This classic type 2 (innate) immune response contributes to anti-helminth immunity, lipid metabolism and to the development of various allergic diseases such as asthma, atopic dermatitis, allergic rhinitis, and chronic rhinosinusitis (12, 13, 38–40). Recently, it was reported that IL33-activated tumor infiltrating ILC2s (TILC2) restrict pancreatic tumor growth. Moreover, IL33 induces the expression of inhibitory checkpoint receptor PD-1 in TILC2s. Antibody-mediated PD-1 blockade leads to TILC2 expansion and activation, resulting in augmented anti-tumor immunity, and enhanced tumor control (41).

Treg Cells

Depending on the tissue and disease setting, a significant proportion of Tregs constitutively express the ST2 receptor (32–37). IL-33/ST2 signaling in Tregs has been shown to promote Treg frequency and immunosuppressive capacity in colitis and tissue injury models as well as graft vs. host disease (35, 42). In cancer, IL33/ST2 signaling in Tregs seems particularly important in colon cancer, where the frequency of ST2-expressing Tregs is higher and ST2-expression is upregulated compared to normal colon tissue. Signaling through the ST2 receptor can increase frequency, activity and migratory potential of Tregs, which is associated with increased colonic tumor burden (43–45). However, there are also studies that demonstrate increased Treg density upon genetic ST2 ablation (34).

Mast Cells

While MCs can confer their functions through cell-cell contacts, their predominant way of shaping their cellular environment occurs via release of preformed or newly synthesized mediators. These paracrine acting molecules include growth factors, proteases, leukotrienes, cytokines and chemokines which in turn modulate biological processes and responses including: tissue remodeling, angiogenesis, pro/anti-inflammatory responses, immunosuppression, and cellular proliferation, survival, recruitment, maturation and differentiation (46, 47).

MCs provide critical nodes for IL33 signaling in innate immune cells. In external surface organs, where epithelial cells express high levels of IL33, the number of MCs is highest (48). MC's are first responders during infections, where IL33 acts as an alarmin following its release as a cellular danger signals (49). The dual importance of IL33 and MCs in allergies is well established (50), yet critical roles for the IL33-MC axis have also been uncovered in allergic, autoimmune, inflammatory disease as well as cancer and other diseases (51, 52). In addition, MCs can potentiate the biological impact of IL33, because chymases and tryptases released by activated MCs process full-length IL33 into a truncated and biologically more active mature protein (53). In addition, MCs have been described to also produce IL33 (7).

MCs appear to be the only cell type which constitutively express high levels of ST2 independent of their tissue origin or maturation/activation status (33, 54). Importantly, activation of MCs by IL33 leads to the release of a plethora of factors that act on various cell types in the TME and influence their recruitment, rate of proliferation and their state of activation, differentiation and polarization (**Figure 1**) (46, 55–65).

The striking overlap of cell types which respond to IL33 and mast cell-released mediators highlights the importance of the IL33-MC axis for the biological outcome and demonstrates the potential of MCs as amplifiers and regulators of IL33-mediated processes. However, most past studies have investigated the roles of IL33/ST2 and of MCs separately. We and others have begun to better integrate these closely related aspects of innate cell biology in the context of GI cancer, since this organ system is known for both high IL33 expression and high density of MCs.

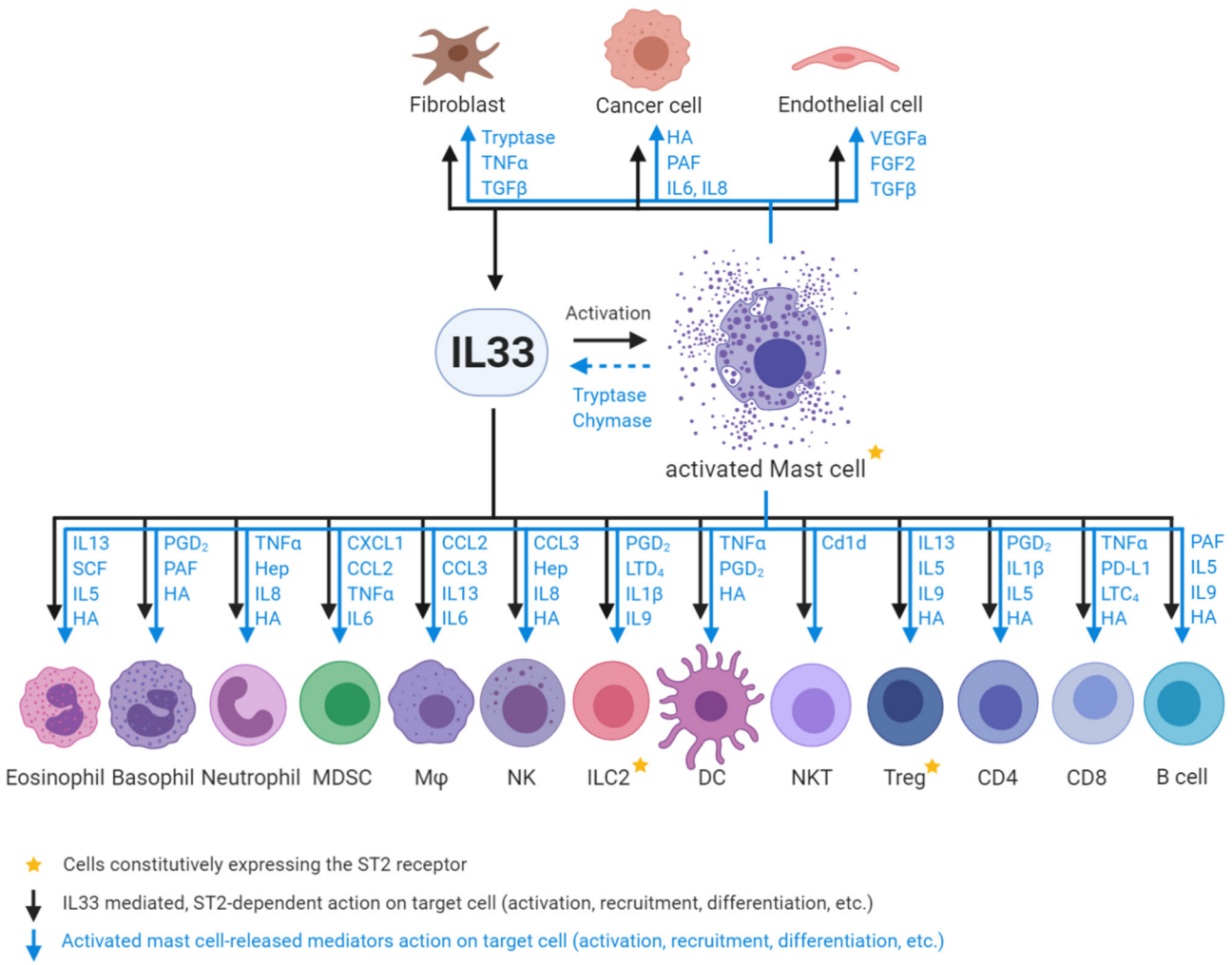

FIGURE 1 | Interactions between IL33, activated MCs and ST2-positive responder cells. Fibroblasts, endothelial and epithelial/tumor cells are the major source of IL33 in the tumor microenvironment, and can in turn be stimulated by IL33. IL33 activates MCs and in turn MC -released chymases/tryptases cleave full length IL33 into highly active mature IL33. Subsequently, both IL33 (via ST2 receptor binding on target cells) and activated MC (via mediator release; depicted in blue) action innate immune cells: eosinophils, basophils, neutrophils, myeloid derived suppressor cells (MDSC), macrophages (Mϕ), natural killer cells (NK), type 2 innate lymphoid cells (ILC2), dendritic cells (DC), and adaptive immune cells: natural killer T cells (NKT), regulatory T cells (Treg), CD4 T cell subsets (Th1/2/17), CD8 T cells (CD8), and B cells. Mast cell mediator abbreviations: TNFα, Tumor necrosis factor alpha; TGFβ, Transforming growth factor beta; HA, Histamine; PAF, Platelet activating factor; IL, Interleukin; VEGFa, Vascular endothelial growth factor A; FGF2, Fibroblast growth factor 2; SCF, Stem cell factor; PGD_2, Prostaglandin D_2; Hep, Heparin; CXCL1, C-X-C-motif chemokine; CCL, C-C motif chemokine ligand; LTD_4, Leukotriene D_4; Cd1d, Cluster of differentiation 1 family glycoprotein; PD-L1, Programmed death-ligand 1; LTC_4, Leukotriene C_4.

Other Cell Types

Besides the constitutively ST2-expressing ILC2, Tregs and MCs, there are various cell types, which don't express ST2 at steady state but expression can be induced or is present in minor cellular subsets. These include endothelial cells (66, 67), epithelial and epithelial-derived cancer cells (68, 69), fibroblasts (34, 70) and other non-immune cell types. Importantly, fibroblasts, endothelial and epithelial cells are also the major cellular sources of IL33 production in the tumor microenvironment (**Figure 1**) (3–5). The immune cells that respond to IL33 in a ST2-dependent manner (in addition to MCs, Tregs and ILC2s) are the innate immune cells: eosinophils, basophils, neutrophils, myeloid derived suppressor cells (MDSC), macrophages (Mϕ), natural killer cells (NK), dendritic cells (DC), and the adaptive immune cells: natural killer T cells (NKT), CD4 T cell subsets (Th1/2/17), CD8 T cells, and B cells (**Figure 1**) (71–80).

IL33 AND MAST CELLS IN GASTROINTESTINAL CANCER

Various reviews try to group the IL33-responding immune cell types based on their role in tumor growth, whereby MCs, (tumor associated) macrophages and Tregs are considered pro-tumorigenic, while CD8, NK, NKT, and DC conferring predominantly anti-tumorigenic functions (6, 74, 77, 81). Beside

these "classical activities," for many of these cells both anti- and pro-tumorigenic roles have been described and for most cell types their functions might be tumor-type and -stage dependent.

The role of IL33 in cancer has been reviewed recently (81, 82). IL33 expression correlates with poor prognosis in some cancers, but predicts good outcomes in others (77). Likewise for MCs, high mast cell infiltration can correlate either with poor or good prognosis depending on the tumor type (65).

Pre-cancerous Inflammation

Chronic inflammation or infection often precedes neoplastic transformation. Accordingly, IL33 expression is elevated in colonic epithelial cells and myofibroblasts of ulcerative colitis patients (83, 84) and in the chronically inflamed stomachs of patients infected with *H. pylori* or during bouts of acute gastritis (85, 86). Meanwhile, increased MC numbers are readily detected in patients with ulcerative colitis, gastritis and various other inflammatory disorders of the GI tract [reviewed in (87)] and have been attributed a disease-promoting role (88).

Conversely, simultaneous ablation of MCP-6/7, mouse orthologs of the human b tryptases TSAB1/2, significantly protected mice from dextran sodium sulfate (DSS)-induced colitis (89). While thi observation suggests that MCs may promote the inflammatory environment that mediates DSS-dependent destruction of the epithelial layer, the role of MC during the subsequent "wound-healing reaction" remains less clear. Although, it has been noted that tryptase-expressing MCs persist for several weeks at the site of the original injury (90). Consistent with a role for MC to not only release various leukocyte attracting chemokines, but to also induce proliferative effects on fibroblasts and other "bystander" cells (91). In turn, soluble factors from fibroblasts, including IL-33 can then feed-forward on MC and shape their phenotype (92). Indeed, in response to DSS administration, IL33 activated MCs in the colonic epithelium, which subsequently promoted restoration of epithelial barrier function and regeneration of epithelial tissues (93). In accordance with this, Rigoni et al. observed exacerbated colitis in MC-deficient Kit^{w-Sh} mice (94). Collectively these preclinical studies suggest a functional connection between IL33 and MCs during inflammation-associated regeneration of the GI epithelium. Similarly, tumors, "wounds that do not heal," may co-opt these wound-healing associated IL33-mast cell immune responses (95).

Intestinal and Colorectal Cancer

Although IL33 is elevated in colorectal cancer (CRC) patients when compared to normal tissues, in some studies its levels were reduced when comparing late vs. early stage disease (70, 96–98). Mast cell infiltration is associated with poor prognosis in colorectal cancer patients [reviewed in (65)], and at least one study also associated high IL33 expression with poor survival outcomes for metastatic CRC (99). Meanwhile, IL33-ST2 mechanisms underpinning pro- and anti-tumoral roles in CRC have been studied in mice. Maywald et al., observed reduced intestinal polyposis in IL33-deficient Apc^{Min} mice, which was associated with a lack of IL33-mediated mast cell and myofibroblast activation (70). A tumor promoting role for IL33 was confirmed independently (44). However, two separate studies reported elevated tumor burden in MC-deficient Apc^{Min} mice when compared to their MC-proficient counterparts (100, 101). Meanwhile, intestinal polyps in $Apc^{\Delta 468}$ mutant mice have increased IL33 expression and reduced numbers of MCs contribute to the anti-tumoral effect of IL10-deficiency (54) and 5-lipoxygenase-deficiency (102).

In the classic carcinogen-induced mouse model of sporadic colon cancer (6x AOM), colon tumors displayed increased expression of IL33 and ST2. However, mast cell numbers were unchanged, while ST2-deficieny increased number and size of the colon tumors. Surprisingly, the tumor suppressive role of the IL33-ST2 signaling pathway occurred independently of MC abundance, but was mediated by mesenchymal (stem) cells and associated with a strong interferon gamma (IFNγ) gene expression signature (34).

However, in the AOM/DSS inflammation-associated CRC model, ST2-deficient mice had reduced tumor burden, possibly owing to ST2-expressing Tregs although these authors neither investigated the number nor activation status of MCs (43). Using the same model, Mertz et al. also observed reduced tumor burden in ST2-deficient mice (98). Using adoptive bone marrow chimeras, these authors attributed the anti-tumor effect to both the radio-resistant and radio-sensitive cell compartments and demonstrated an involvement of several hematological cell types (98). The latter observation was consistent with earlier work demonstrating reduced colonic tumor burden in MC-deficient c-Kit^{W-sh} mice following the AOM/DSS challenge (94).

Gastric Cancer

IL33-mediated spasmolytic polypeptide-expressing metaplasia (SPEM) in the stomach of mice is associated with a strong Th2 cytokine response, suggesting an involvement of MCs (103). In human gastric cancer cell lines, IL33 promoted epithelial-to-mesenchymal transition *in vitro* and xenograft tumor growth in an ST2-dependent manner (104). Recently, we illustrated that MC numbers are elevated in human gastric cancer specimens and that high expression of an IL33-MC activation gene signature predicts poor survival of intestinal-type gastric cancer in patients (33). Utilizing mouse models, we identified an IL33-MC-macrophage axis promoting gastric cancer growth where either ST2-deficiency, lack of MCs or lack of macrophages all restricted gastric cancer growth in the preclinical $gp130^{FF}$ mouse model of inflammation-associated gastric cancer. IL33-mediated activation of MCs and subsequent secretion of macrophage attracting factors form part of a mechanism resulting in the accumulation of pro-angiogenic and pro-tumorigenic macrophages in the gastric tumors. In ST2-deficient $gp130^{FF}$ mice, ILC2 and Treg density was not altered, while frequency of MCs was decreased and associated with reduced tumor growth. Conversely, adoptive transfer of ST2-proficient MC stimulated tumor growth in ST2-deficient $gp130^{FF}$ mice, demonstrating that IL33-ST2 signaling within MCs is part of the tumor promoting effect of IL33 in gastric cancer (33).

Other Cancers of the Gastrointestinal Tract

IL33 administration promoted the growth of Kras and TGFbR2 mutant biliary tract cancers (105) and in mouse models with constitutively active AKT/YAP pathway (106, 107). Moreover, IL33 is overexpressed in human gallbladder cancer patients (108). However, in pancreatic cancer patients high IL33 expression and high number of tumor-infiltrating ILC2s correlated with better survival (41). This is consistent with the observation in a pancreatic cancer mouse model, that IL33 activated tumor–associated ILC2s mediated anti-tumor immunity. MCs were not investigated in this study, even though MC's pancreatic tumor promoting functions are known (109). Finally, IL33 is highly expressed in patients with esophageal squamous cell carcinoma. In corresponding cell lines, IL33 overexpression promoted migration and invasiveness, while IL33 knockdown inhibited the metastatic potential of these cells (110).

THERAPEUTIC TARGETING OF THE IL33-MC AXIS

In recent years, a number of studies have identified compounds that inhibit IL-33 mediated activation of MCs. Amongst those are natural compounds from plants like berberine (111), methoxyluteolin (112), and resveratrol (113) or ES-62 produced from parasitic worms (114) as well as various other drug classes including didox (synthetic ribonucleotide reductase inhibitor) (115), chondroitin sulfate (glycosaminoglycan) (116), triochastatin A (histone deacetylase inhibitor) (117) and the growth factor TGFb1 (118). However, in all these studies, drug effects were investigated exclusively *in vitro*. *In vivo* testing in preclinical animal models is required to increase the impact of these findings and investigate their IL33-MC axis specificity and potential off-target effects.

A promising example for an unbiased high-throughput approach to identify IL33-MC modulating drugs was published by Ramadan et al., They conducted a high-throughput screen of over 70,000 small molecules utilizing an AlphaLISA assay, which measures ST2-Fc fragment binding to IL33 (119). The lead compounds were then demonstrated to exhibit activity *in vitro* as well as *in vivo* in mouse models for graft vs. host disease.

Targeting IL33/ST2

Development and characterization of inhibitors of IL33-ST2 signaling is an active field of research. Various synthetic molecules, antibodies and natural compounds either targeting the IL33-ST2 interaction directly, or inhibiting MyD88-IRAK and other downstream signaling pathways, or disrupting production of mediators are in now pre-clinical testing (74).

Targeting the IL33-ST2 interaction strategies are favored due to the knowledge gained from the naturally occurring soluble ST2 receptor isoform (sST2), a secreted "decoy receptor," which binds IL33 and thereby sequestering the ligand from binding to membrane-bound ST2. High sST2 expression has been associated with anti-tumor responses in several cancers (120). However, the most advanced modalities targeting the IL33-ST2 interaction are antibodies, with five different anti-IL33 or anti-ST2 antibodies being tested in clinical phase 1 trials and found to be safe for use in humans (NCT02170337, NCT01928368, NCT02958436, NCT02999711, NCT03112577, NCT02345928, NCT03096795). Currently, there are multiple phase 2 trials ongoing/completed investigating the efficacy of IL33-ST2 inhibition against various allergic and inflammatory diseases and diabetic kidney disease (**Table 1A**).

To date, no clinical trials have been conducted in cancer patients. Indeed, only a limited number of studies have used IL33-ST2 neutralizing antibodies in preclinical tumor models *in vivo* (**Table 1B**). Strikingly, all these studies demonstrated anti-tumor effects of anti-IL33 and anti-ST2 antibody treatments. However, as a cautionary tale, multiple studies demonstrate anti-tumor effects upon administration of recombinant IL33 (34, 41).

Targeting MCs

A plethora of strategies to target MC receptors, intracellular signaling components and MC-derived mediators have been tested, with some now being used in the clinic. Traditionally, agents targeting MCs were studied and applied in allergies and related disorders (129, 130). Accordingly, mast cell stabilizers, drugs like Cromolyn sodium, Nedocromil, and Lodoxamide, which block MC degranulation are utilized for indications like asthma and other allergic diseases (130).

A number of tyrosine kinase inhibitors including Nilotinib, Sunitinib, Dasatinib, Imatinib, and Masitinib are in clinical trials or in clinical practice as anti-cancer drugs (130). All these small molecule inhibitors have high affinity for the tyrosine kinase receptor KIT, in addition to other tyrosine kinases. KIT is a key molecule for MC development, proliferation, survival and function and inhibition of KIT reduces MC numbers and inhibits their function. For example, Imatinib was shown to reduce asthma symptoms in a MC-dependent manner (131), yet the impact of these TK inhibitors on MCs and their contribution to the anti-tumor effect has not been investigated systematically. In the first instance, it would be important to establish whether tumors with high MC numbers respond better to anti-KIT tyrosine kinase inhibitors.

The field of targeting IL33-ST2 signaling is quickly progressing, with neutralizing antibodies being the most promising agents. While these antibodies advance rapidly in clinical trials for various inflammatory disorders, their use as anti-cancer agents is only just beginning. More work is required to better dissect tumor-promoting from tumor suppressing roles conferred by the IL33-ST2 axis in order to predict in which tumor microenvironment inhibition of IL33-ST2 signaling or MCs will be beneficial.

CHALLENGES FOR THE FIELD

The importance of IL33 and MCs in GI cancer has been well documented. In recent years, there has been some progress in understanding the mechanisms of how the IL33-MC axis acts

TABLE 1A | Clinical trials utilizing antibodies targeting IL33/ST2.

Antibody	Company	Clinical trial	Phase	Indication	Status/Results
MSTT1041A,	Genentech/	NCT02918019	2b	Uncontr. severe asthma	Completed
AMG 282, RG6149 (anti-ST2)	Amgen	NCT03747575	2	Atopic dermatitis	Active, not recruiting
REGN3500,	Sanofi/Regeneron	NCT03387852	2	asthma	Completed, met 1st & 2nd endpoint
SAR440340		NCT03546907	2	COPD*	Recruitment completed
(anti-IL33)		NCT03736967	2	Atopic dermatitis	Recruiting
		NCT03738423			
GSK3772847,	GSK/	NCT03207243	2a	Severe asthma	Recruitment completed
CNTO 7160 (anti-ST2)	J&J	NCT03393806	2	Asthma with AFAD*	Active, not recruiting
ANB020,	Anaptysbio	NCT02920021	2	Peanut allergy	Completed (121)
Etokimab		NCT03469934	2	Eosinophilic asthma	Recruitment completed
(anti-IL33)		NCT03533751	2	Atopic dermatitis	Completed (122)
		NCT03614923	2	Chron. Rhinosinusitis with NP*	Recruiting
MEDI3506	AstraZeneca	NCT04170543	2a	Diabetic kidney disease	Recruiting
(anti-IL33)		NCT04212169	2	Atopic dermatitis	Recruiting

**COPD, chronic obstructive pulmonary disease; *AFAD, allergic fungal airway disease; *NP, Nasal Polyps.*

TABLE 1B | Studies utilizing antibodies IL33/ST2 in tumor models in mice.

Reference	Antibody	Cancer model	Result	MCs analyzed
Guabiraba et al. (123)	Anti-IL33, anti-mouse, clone 396118, MAB3626, R&D	CT26 colon cancer cell line subcutaneous	aIL33+Irinotecan -> anti-tumor effect	No
Nakagawa et al. (105)	Anti-IL33, R&D	KTC-K19CreERT extrahepatic cholangiocarcinoma mice	Anti-tumor effect	No
Wu et al. (124)	Anti-IL33, anti-human, MAB36254, R&D	Renal cancer cell lines 786O and OSRC2 subcutaneous in nude BalbC	Anti-tumor effect	No
	Anti-ST2, anti-human, Clone MAB523, R&D		Anti-tumor effect	No
Zhou et al. (125)	Rabbit anti-mouse, R&D	CT26 colon cancer cell line subcutaneous	Anti-tumor effect	No
	Rabbit anti-mouse, R&D		Anti-tumor effect	No
Kim et al. (126)	Anti-ST2, anti-mouse, clone 245707, MAB10041, R&D	KrasG12DxCCSP-Cre lung cancer model	Anti-tumor effect	No
Lin et al. (127)	Anti-ST2, monoclonal anti-human, R&D	Ln229 glioma cell line subcutaneous in NSG mice	Anti-tumor effect	NSG are MC-def.
Maywald et al. (70)	Anti-ST2, mu-IgG1-FC–anti-muST2, Amgen	ApcMin intestinal cancer model	Anti-tumor effect	Yes, MC number + activation decreased in IL33KO/anti-St2 treated tumors
Kudo-Saito et al. (128)	Anti-IL33, anti-mouse, R&D	B16F10 melanoma subcutaneous and intravenous	Anti-tumor	Yes, MC increased in BM metastasis

in GI cancers. While there is an increasing interest in targeting this signaling node in various diseases, the few drug candidates currently undergoing clinical testing have not been utilized in cancer trials yet. This is due to the dichotomous actions of IL33 and MCs in cancer. Below we dicuss some of the aspects of IL33 and MC biology which need to be addressed in order to advance the field toward harnessing IL33/MCs targeting as a novel treatment option for GI cancers.

Diversity of Cell Types Responding to IL33

While there is now ample evidence that the IL33-MC axis is important for many cancers, the multitude of cell types in the TME able to respond to IL33 and mediated either pro- or anti-tumorigenic effects presents a formidable challenge for predicting the outcome of anti-IL33/anti-ST2 therapies. We propose that a detailed investigation of the spatial distribution of IL33-expressing cells and ST2-presenting responder cells

in combination with full immunophenotyping of tumors will help addressing these issues. Since oxidation of IL33 in the extracellular space occurs rapidly and drastically reduces its ability to bind ST2 and trigger downstream signaling activation (132), we speculate that only the ST2-expressing cells in close spatial proximity of IL33-rpoducing cells will respond to IL33. Novel technologies like multiplex immunofluorescence microscopy, will allow spatial identification of cell types expressing IL33 and ST2, enabling prediction of responder cell types. Because Tregs and ILC2s are also constitutively expressing ST2, these cell types should be the included in studies attempting to predict anti-tumor effects of IL33-ST2 inhibition.

Also, further research is required to better understand the temporal dimension of IL33 secretion and the cell types responding during early vs. late stages of tumorigenesis. Indeed some studies suggest that IL33 expression is decreased in more advanced disease (97, 98) while serum levels of IL33 increased in patient with advanced gastric cancer (133). Tissue resident ST2-expressing cells, like MCs and ILC2s are the dominant IL33 responders during the early stages of tumor development. However, it is not known whether these cells can lose their responsiveness to IL33 in the changing tumor microenvironment, for example, by downregulating expression of ST2, nor has it has been investigated whether the dominant IL33 responses shift with increasing tumor size and progression of disease toward ST2-positive cells newly recruited into the tumors. Nevertheless, there is significant evidence of the role of MCs and IL33 in late stage cancers, particular in the context of tissue remodeling, epithelial to mesenchymal transition and invasion (104, 128, 134, 135).

MC Heterogeneity

Many effects of IL33 are mediated through MC activation. However, the true extent of MC heterogeneity within the TME is not well understood. Only a few whole transcriptome studies are published, all of them were performed on bulk MCs isolated from healthy mice or humans. As part of the FANTOM5 project, Motakis et al. (136) elucidated the transcriptome of human skin MCs and compared against *ex vivo* cultured MCs. They found MC-specific gene signatures distinguishing the skin MCs from various other cell types, and discovered significant changes in gene expression profiles suggesting significant de-or trans-differentiation associated with *in vitro* propagation of MCs cultured (136). This warrants careful interpretation of findings obtained from *in vitro* studies. Transcriptional profiling of MCs from various tissues against other major immune cell lineages, revealed not only distinct differences between the various cell types but also considerable transcriptional heterogeneity between MCs recovered from different tissues (137). Indeed, a recent review suggested to replace the currently used system of histological classification of MCs with a system based on MC protease expression to more accurately reflect the tissue-specific versatility of MCs (138). Single cell sequencing studies of cancer-associated MCs are required to elucidate the true extent of mast cell heterogeneity to better understand the various biological consequences of mast cell activation in the cancer setting.

Diversity of Mast Cell Activation Signals

Following on from the initial study by Schmitz et al., the ability of IL33 to activate MCs has been studied extensively (2, 139). However, MCs are key sentinel cells that express many receptors on their surface (46, 140), resulting in a multitude of environmental factors able to trigger their activation.

Allergen IgE-mediated activation of MCs was the first to be identified and is well characterized in the context of allergic pathologies, yet many other factors can activate MCs in an IgE-independent manner (52, 139).

Numerous studies have shown that IL33-elicited responses in MCs differ from IgE stimulation and that IL33-mediated responses in MCs are modified, and often potentiated, when secondary stimuli like IgE, substance P or IL3 are present (112, 141–144). Further research is required to uncover other MC-activating factors present in the tumor microenvironment and how they impact IL33 signaling and MC activation.

CONCLUSIONS

Diverse functions for both IL33 and mast cells were uncovered in the context of cancer initiation and progression. However, only by focusing on the IL33/MC axis, rather than studying these key regulators of immunity separately, and by utilizing novel technologies, will the full potential of targeting IL33 signaling and MC activation be discovered and exploited for anti-cancer therapies.

AUTHOR CONTRIBUTIONS

MFE, MB, and ME: conception, design, writing, reviewing, and editing of the manuscript. All authors: contributed to the article and approved the submitted version.

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REVIEW
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Is There a Role for Basophils in Cancer?

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Basophils were identified in human peripheral blood by Paul Ehrlich over 140 years ago. Human basophils represent <1% of peripheral blood leukocytes. During the last decades, basophils have been described also in mice, guinea pigs, rabbits, and monkeys. There are many similarities, but also several immunological differences between human and mouse basophils. There are currently several strains of mice with profound constitutive or inducible basophil deficiency useful to prove that these cells have specific roles *in vivo*. However, none of these mice are solely and completely devoid of all basophils. Therefore, the relevance of these findings to humans remains to be established. It has been known for some time that basophils have the propensity to migrate into the site of inflammation. Recent observations indicate that tissue resident basophils contribute to lung development and locally promote M2 polarization of macrophages. Moreover, there is increasing evidence that lung-resident basophils exhibit a specific phenotype, different from circulating basophils. Activated human and mouse basophils synthesize restricted and distinct profiles of cytokines. Human basophils produce several canonical (e.g., VEGFs, angiopoietin 1) and non-canonical (i.e., cysteinyl leukotriene C_4) angiogenic factors. Activated human and mouse basophils release extracellular DNA traps that may have multiple effects in cancer. Hyperresponsiveness of basophils has been demonstrated in patients with $JAK2^{V617F}$-positive polycythemia vera. Basophils are present in the immune landscape of human lung adenocarcinoma and pancreatic cancer and can promote inflammation-driven skin tumor growth. The few studies conducted thus far using different models of basophil-deficient mice have provided informative results on the roles of these cells in tumorigenesis. Much more remains to be discovered before we unravel the hitherto mysterious roles of basophils in human and experimental cancers.

Keywords: angiogenesis, angiopoietins, basophil, cancer, cysteinyl leukotrienes, cytokines, vascular endothelial growth factors

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INTRODUCTION

Peripheral blood basophils and tissue mast cells were described over 140 years ago by Paul Ehrlich the founder of modern Immunology (1, 2). Basophils have been characterized in humans (3), guinea pigs (4), mice (5, 6), rabbits (7) and monkeys (8). Basophils represent <1% of human peripheral leukocytes, whereas mast cells are ubiquitous in essentially all tissues (9, 10). Basophils share some characteristics with mast cells, including the presence of similar, but distinctive basophilic granules within the cytoplasm (11), surface expression of the full tetramer ($\alpha\beta\gamma_2$) form of the high affinity receptor for IgE (FcεRI) and release of proinflammatory mediators such as histamine and cysteinyl leukotrienes (12, 13). These similarities had initially generated the erroneous hypothesis that basophils represented the circulating precursor/counterpart of tissue mast cells. This concept is no longer accepted, as there is now ample evidence that human basophils and mast cells differ morphologically, ultrastructurally, immunologically, biochemically, and pharmacologically (13–15). In a series of eloquent studies, Ann M. Dvorak carefully described and compared the distinctive morphological and ultrastructural features of human basophils and mast cells (11). **Figure 1** illustrates the striking ultrastructural differences between human peripheral blood basophils and lung mast cells (18). In addition to highlighting key ultrastructural differences between basophils and mast cells, Dr. Dvorak also pioneered the characterization of mouse basophils. In fact, there was early belief that questioned the existence of basophils in mice. However, Dr. Dvorak's meticulous work clearly identified mouse basophils as a rare, and often elusive, population of granular cells typically found in bone marrow, with some ultrastructural characteristics similar to human basophils (6, 11, 19).

BASOPHIL DEVELOPMENT

Like other myeloid lineages basophils develop from hematopoietic stem cells in the bone marrow (20). IL-3 is generally viewed as the most important growth factor for basophil development, both in humans and mice (21, 22). Indeed, human and murine basophils can be generated *in vitro by* culturing bone marrow cells in the presence of recombinant IL-3 (23–25). More recently, it has been proposed that thymic stromal lymphopoietin (TSLP) is another growth factor important for the development of mouse basophils (26). Interestingly, IL-3- and TSLP-elicited murine basophils differ in terms of gene expression and functions, suggesting heterogeneity among these basophil populations (27). A study has suggested clinical relevance to this concept in reporting evidence that a small percentage (? 10%) of basophils isolated from asthmatic patients express the TSLP receptor and respond directly to TSLP by releasing histamine and cytokines (28). In contrast, subsequent studies have failed to confirm these findings, showing that human basophils lack expression of the IL-7Rα subunit of TSLP receptor (29) and are unresponsive to *in vitro* stimulation with TSLP (29, 30). Collectively, these findings illustrate some of the controversies yet to be resolved between human and mouse basophils, but also those within each species (13, 31, 32).

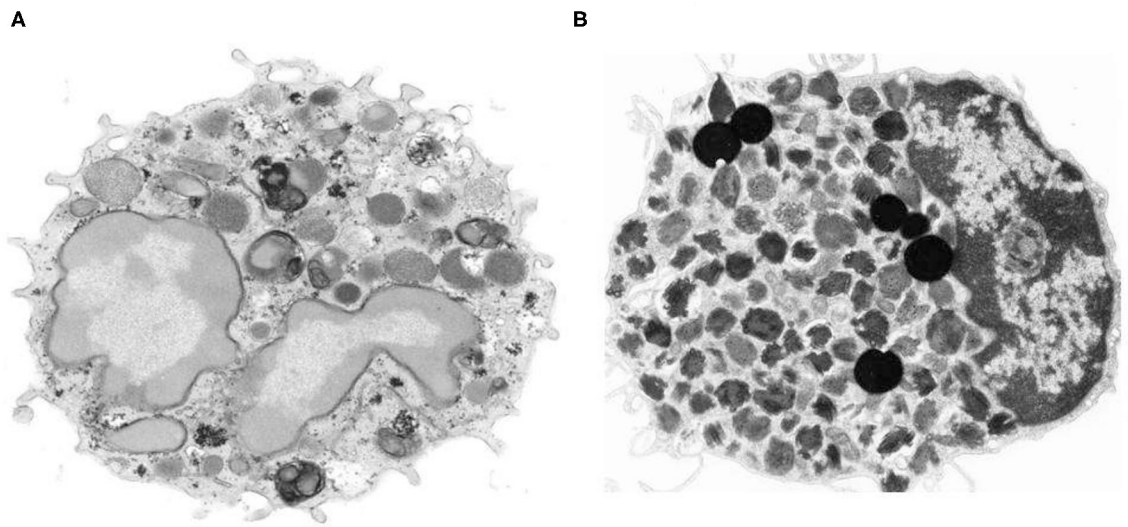

FIGURE 1 | Morphologic and ultrastructural differences between human basophils and mast cells. **(A)** Human peripheral blood basophil shows irregular blunt surface processes and a polylobed nucleus with condensed chromatin pattern. The cytoplasm contains large-membrane bound secretory granules filled with electron dense particles and/or finely granular material (11) X 21,500. **(B)** Isolated human lung mast cell has a narrow surface fold and single lobed nucleus with partially condensed chromatin pattern. The cytoplasm is filled with a large number of membrane-bound secretory granules that have an extremely variable ultrastructural pattern (16, 17). The cytoplasm also contains six non–membrane-bound spherical lipid bodies that are larger than secretory granules, are osmophilic and do not contain scrolls (16, 17) X 14,000. Photos kindly provided by Ann M. Dvorak and reproduced with permission from Marone et al. (18).

PROINFLAMMATORY AND IMMUNOREGULATORY MEDIATORS/CYTOKINES RELEASED BY BASOPHILS: HUMAN *VS.* MOUSE

Many phenotypic markers have been identified on human and mouse basophils, with some minor differences worth noting. For example, basophils from both species express of a variety of activation-linked markers, namely FcεRI (33, 34), but also the degranulation marker, CD63 (35–37), as well as CD203c – an ecto-nucleotide pyrophosphatase/phosphodiesterase (15, 36, 38, 39). In contrast, human basophils express the IgG receptors FcγRIIA, FcγRIIB, and minute amounts of FcγRIIIB, whereas mouse basophils express FcγRIIB and FcγRIIIA (40, 41). As indicated above, both human and mouse basophils express receptors for IL-3 (CD123) (26, 42), but also for GM-CSF (CD116) (43), and IL-33 (ST2) (44–47). Again, it remains unclear whether they similarly express the heterodimeric receptor for TSLP (26, 28–30). To date, only human basophils are reported to express IL-5 receptors (CD125). Human basophils express tropomyosin receptor kinase A (TrkA)(48, 49)—the high affinity receptor for nerve growth factor (NGF) and that this factor mediates functional activity (50). In contrast, there are currently no reports that mouse basophils express TrkA. Both human and mouse basophils share the expression of a variety of chemokine receptors (13, 51–56), but it remains to be determined if mouse basophils express CCR1 and CXCR1 (57). These phenotypic comparisons between human and mouse basophils are summarized in **Table 1**.

There are several proinflammatory mediators found preformed in human basophils, including histamine ($\simeq$ 1 pg/cell), basogranulin (57, 77) and very low concentrations of tryptase (78). Human (79) and mouse basophils release granzyme B (80), which possesses cytotoxic effects on cancer cells (81, 82). Both human and mouse basophils rapidly synthesize cysteinyl leukotriene C_4 (LTC_4) through the 5-lipoxygenase pathway (83). There is evidence that mouse basophils metabolize arachidonic acid through cyclooxygenase activity to form prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) (72, 84). In contrast, there is currently no solid evidence that highly purified human basophils can produce measurable levels of PGD_2, or any other lipid mediator generated through the cyclooxygenase pathway (85).

With regard to the cytokines secreted by human *vs.* mouse basophils, there are several similarities and differences. First, it is now well-accepted that both human and mouse basophils produce IL-4 (44, 86–97) and IL-13 (44, 89, 92, 94, 97–100). Several reports show that mouse basophils additionally produce IL-6 (44, 73, 101) and TNF-α (44, 73). There are at least two publications reporting TNF-? production by human basophils (88, 102). Numerous attempts to detect this cytokine in supernatants of highly purified human basophils activated by IgE-mediated stimuli have produced negative results. Certainly, other cell types (e.g., monocytes, DCs) produce copious amounts of TNF-? and IL-6 (103, 104), thus making it possible that even low-level contamination with these cells could skew the basophil findings. This issue must be taken into consideration each time any cytokine is reportedly made by basophils. Nevertheless, consistent with the general theme of this review, it is becoming apparent that basophils secrete several angiogenic factors that, when combined with the cytokines thus far mentioned, point to a possible role for these cells in wound healing and/or tumorigenesis (as further discussed below). In particular, vascular endothelial growth factor-A (VEGF-A) (57), angiopoietin-1 (ANGPT1) (105), hepatocyte growth factor (HGF) (44, 106), and amphiregulin (71, 107, 108) are all reportedly produced by human basophils, with some of these also made by

TABLE 1 | Comparison of the phenotypic differences between human and mouse basophils[a,b].

Phenotypic Marker	Human Basophil	Mouse Basophil	References
FcεRI	++	++	(34)
FcγRIIA	+	-	(33, 40, 58)
FcγRIIB	+	+	(33, 40, 58)
FcγRIIIA	-	+	(33, 40, 58, 59)
FcγRIIIB	±	-	(33, 40, 58, 60)
CD63	+	+	(35–37)
CD203c	+	+	(15, 36, 38, 39)
(CD123) IL-3Rα	++	++	(26, 42)
(CD116) GM-CSFRα	+	+	(43)
(CD125) IL-5Rα	+	ND	(43)
TSLPR	–	+	(26, 28–30, 32)
(ST2) IL-33R	+	+	(44–47)
CCR1	+	ND	(13, 51)
CCR2	++	+	(13, 51–53)
CCR3	++	±	(13, 51, 61)
CCR5	+	–	(13, 51, 53)
CXCR1	++	ND	(13, 51)
CXCR2	+	+	(13, 51, 62)
CXCR4	+	+	(13, 51, 62, 63)
CRTH2	++	+	(51, 55, 62, 64, 65)
CD200R	+	+	(56, 66)
CD300a	+	+	(67–69)
CD300c	+	+	(68, 70)
CD300f	+	+	(68, 70)
PD-L1	+	ND	(50)
VEGFR2	+	ND	(57)
NRP1/2	+	ND	(57)
TrKA	+	ND	(48, 49)

ND, not done.

[a] Several key surface markers are used to characterize human [IgE$^+$, FcεRI$^+$, CCR3$^+$, (CD123)IL-3Rα$^+$, CD63$^+$, CD203c$^+$] (15, 36, 57, 61, 71) and murine basophils (FcεRI$^+$, KIT$^-$, CD49b$^+$, CD200R3$^+$) (35, 62, 72–76) by flow cytometric analysis.

[b] This table essentially includes the phenotypic characteristics of peripheral blood human and mouse basophils. Phenotypic and/or molecular characteristics of human (50) and mouse basophils in tissues (26, 39, 44, 53, 55, 62) are also included.

+: means "expressed"; ++: means "highly expressed"; – means "not expressed"; ±: means "probably expressed under certain circumstances".

TABLE 2 | Comparison of the mediators differently produced by human and mouse basophils.

Mediator		Human Basophil	Mouse Basophil	References
Cytokines	IL-3	+	+	(21, 109)
	IL-4	++	+	(44, 87–91, 93–97, 110, 111)
	IL-5	–	ND	(86)
	IL-6	±	+	(44, 73, 89, 95, 101)
	IL-8/CXCL8	+	ND	(86, 88–90, 112)
	IL-13	+	+	(44, 88, 89, 92, 94, 97–100)
	IL-31	+	+	(113)
	TNF-α	±	+	(44, 73, 88, 95, 102)
Chemokines	CCL3	+	+	(100, 114)
	CCL5	+	ND	(112)
	CXCL10	+	ND	(112)
Angiogenic factors	VEGF-A	+	ND	(57)
	VEGF-B	+	ND	(57)
	ANGPT1	+	ND	(105)
	HGF	+	+	(44, 106)
	LTC_4	+	+	(83, 115)
	Amphiregulin	+	+	(71, 107, 108)
Extracellular DNA Traps		+	+	(116–118)
Granzyme B		+	+	(78, 79)

ND, not done.
+: means "expressed"; ++: means "highly expressed"; – means "not expressed"; ±: means "probably poorly expressed".

mouse basophils (44). **Table 2** summarizes the cytokines/factors produced by human *vs.* mouse basophils.

There are many other fundamentals of basophil biology not discussed herein, but have been extensively reviewed elsewhere (13, 86, 119–123). In this review, we focus our discussion instead on the relatively novel concept of how basophils and their mediators/cytokines may play a role in promoting or limiting tumorigenesis.

DIFFERENCES BETWEEN PERIPHERAL BLOOD AND TISSUE BASOPHILS

The life-span of peripheral blood basophils has been calculated to be relatively short (? 2.5 days in mice) (124) and therefore newly generated basophils are constantly supplied from the bone marrow to the blood (20). It has long been thought that basophils circulate in peripheral blood and are rarely present in tissues unless during specific kinds of inflammation, reported both in mice (62, 73, 124–126) and in humans (50, 127–131). However, this dogma has been recently challenged by a study in mice whereby the authors found that basophils are present in all phases of lung development (44). Lung-resident basophils localize in close proximity of alveoli and, interestingly, exhibit a specific phenotype, highly divergent from peripheral blood basophils. IL-33 and GM-CSF produced in the pulmonary environment mediate the specific gene signature of lung alveolar basophils. Importantly, lung basophils are essential for transcriptional and functional development of alveolar macrophages and their polarization toward the M2 state. The latter finding raises the intriguing possibility that in pathologies characterized by M2 macrophages, as happens in many tumors (132, 133), basophils may be involved in regulating the activity of tumor-associated macrophages. This experimental study has several relevant pathophysiological implications. First, it demonstrates that tissue resident basophils exhibit a specific phenotype, different from circulating basophils. Second, the tissue microenvironment can modulate the specific gene signature of resident basophils through exposure to cytokines (e.g., IL-33, GM-CSF). Third, lung resident basophils can influence the transcriptional and functional development of macrophages. The observations of this elegant study represent important premises for future research.

We would like to suggest that any difference between circulating and tissue basophils should be confirmed in human models, given the differences between human and murine basophils. Moreover, studies are urgently needed to characterize the possible roles of tissue basophils residing in the tumor microenvironment (TME) of different human tumors in order to identify novel potential prognostic biomarkers and therapeutic targets.

CANONICAL AND NON-CANONICAL ANGIOGENIC FACTORS PRODUCED BY BASOPHILS

Angiogenesis, the formation of new blood vessels from preexisting ones *via* a process called sprouting, represents one of the hallmarks of cancer (134, 135). Angiogenesis is a highly complex process that may occur under physiological conditions, such as during embryonic development. Pathological angiogenesis can occur in inflammation and in cancer and is driven by the coordinated overexpression of several proangiogenic factors (136). Unlike wound healing, where angiogenesis undergoes a resolution phase, tumor angiogenesis continues abnormally in growing cancers supported by angiogenic factors produced by both cancer cells and infiltrated immune cells (137, 138). The VEGF family (VEGF-A, VEGF-B, VEGF-C, VEGF-D) and their receptors (VEGFR1, VEGFR2, VEGFR3) play intricate roles in initiating and promoting tumor and inflammatory angiogenesis (136). Activated human basophils release substantial amounts of VEGF-A, the most potent proangiogenic molecule (57). VEGFs are potent chemotactic stimuli for human basophils through the engagement of VEGFR2expressed in these cells (57, 139). Thus, VEGFs produced by tumor cells and by several immune cells in TME (136, 139–141) can induce basophil chemotaxis through the activation of VEGFR2 on their surface.

The angiopoietin/Tie receptor system is another player in tumor angiogenesis. Angiopoietins (ANGPTs) are a group of growth factors that are involved in regulating vascular functions (142). ANGPTs and their receptors (Tie1 and Tie2) participate in inflammatory and tumor angiogenesis (143). ANGPT1 binds with high affinity to the Tie2 receptor on endothelial cells and promotes endothelial stabilization (144). By contrast, ANGPT2, released by activated endothelial cells, causes vascular permeability. Human basophils constitutively express ANGPT1 and ANGPT2 mRNAs (105). *In vitro* basophil activation causes the release of ANGPT1. Hepatocyte growth factor (HGF) is one of the most powerful angiogenic factors (145) and human basophils are a major source of HGF (106). Recently, it has been demonstrated that mouse lung-resident basophils express a specific gene signature including *Hgf* (44).

The cysteinyl leukotrienes (cys-LTs) are lipid mediators initially characterized for their proinflammatory activities (146). The cys-LTs include leukotriene C_4 (LTC_4), LTD_4, and LTE_4. LTC_4 is *de novo* synthesized by several immune cells (146, 147) and is the major lipid mediator produced by activated human basophils (83, 115). LTC_4 is converted by the extracellular enzymes, γ-glutamyl transpeptidases to LTD_4 and to LTE_4 by the membrane-bound dipeptidases (146). Cys-LTs activate three distinct receptors (CysLTRs) $CysLT_1R$, $CysLT_2R$, and $CysLT_3R$ (148–150). Recent evidence demonstrates that LTC_4 and LTD_4 were equipotent in forming tubes in the Matrigel *in vitro* assay of angiogenesis (151). The proangiogenic activities of LTC_4 and LTD_4 were also confirmed *in vivo* and were found to be mediated by the engagement of $CysLT_2R$ on blood endothelial cells (BECs). $CysLT_2R$ deficiency and pharmacologic antagonism reduced tumor growth and the formation of lung metastases in a mouse model of Lewis lung carcinoma (151). These novel findings emphasize the importance of cys-LTs as non-canonical angiogenic factors in cancer. It is possible to speculate that LTC_4 released by circulating basophils can activate $CysLT_2R$ overexpressed in tumor BECs (151), thus contributing to angiogenesis. It has been suggested that $CysLT_2R$ might represent a possible pharmacologic target in tumor growth and metastases formation (151).

FORMATION OF EXTRACELLULAR DNA TRAPS BY BASOPHILS

Extracellular traps (ETs) are DNA structures released by activated immune cells, including neutrophils, eosinophils, mast cells, macrophages, and basophils (116, 117, 152–155). ETs released by these cells are draped with proteins from primary granules (e.g., myeloperoxidase and elastase) (156), secondary granules (e.g., lactoferrin and pentraxin 3) (156, 157), and tertiary granules (e.g., matrix metalloproteinase 9) (156). Initial studies highlighted the antibacterial activity of ETs (154, 158, 159). During the last years, there has been increasing evidence that ETs, particularly neutrophil extracellular traps (NETs), have a role in different aspects of cancer (160). For instance, it has been demonstrated that NETs can promote cancer metastasis in mouse models and in humans (161–164). Moreover, it has been found that NETs formed during lung inflammation awaken dormant cancer cells (165). Neutrophils from patients with myeloproliferative neoplasms associated with $JAK2^{V617F}$ somatic mutation have an increase in NET formation and thrombosis and mice with knock-in of $JAK2^{V617F}$ have an increased propensity for NET formation and thrombosis (166). Recently, we have demonstrated that anaplastic thyroid cancer cells can induce the release of mitochondrial DNA traps by viable neutrophils (167). Collectively, these studies indicate that NETs can sustain several aspects of tumor growth, the formation of metastasis, and promote cancer-associated thrombosis. Activated human and mouse basophils can form extracellular DNA traps (BETs) *in vitro* and *in vivo* (116–118). Future studies should investigate whether BETs modulate tumor growth and the formation of metastasis in preclinical models and/or in human cancer.

BASOPHIL-DEPLETED MICE TO INVESTIGATE BASOPHIL FUNCTIONS *IN VIVO*

It seems pertinent to review the mouse models currently employed to investigate basophil functions *in vivo*. Basophil-depleted mice will certainly play a critical role in discerning the functions of this granulocyte in cancer. Indeed, several models of basophil-deficient mice have been developed and are undergoing testing for this very purpose.

Initially, studies were performed using administration of antibodies that transiently deplete basophils. These antibodies recognize either the FcεRI (MAR-1) (168) or the activating

receptor CD200R3 (Ba103) (169). Although these antibodies can deplete basophils, they can also deplete/activate other cells (e.g., mast cells, DCs, monocytes) expressing FcεRI (169–171). Furthermore, Ba103 is FcR-dependent and might activate myeloid cells and NK cells (168). Studies using these depleting antibodies have led to the controversial conclusion that basophils have a role as antigen-presenting cells (APCs) during Th2 polarization (95, 172, 173). Several new mouse strains with constitutive or inducible depletion of basophils have recently been generated (119). The Bas-TRECK and the *Mcpt8*^{DTR} are two diphtheria toxins (DT)-inducible basophil depletion mice models (125, 174). The latter models are characterized by a transient depletion of more than 90% of basophils. The *Mcpt8*^{DTR} mice express the human diphtheria toxin (DT) receptor (DTR), which makes it possible to induce a transient ($\sim$ 5 days) depletion of basophils after intraperitoneal treatment with DT (125). The *Mcpt8* gene is specifically expressed by basophils (175, 176) and encodes mouse mast cell protease 8 (mMCP-8), a granzyme B-like protease stored in the secretory granules of basophils (175). Although the expression of Mcpt8 is specific to basophils among mature cells, it is still transiently expressed at the progenitor stage to a sufficient level to allow their depletion by a high dose of DT in the *Mcpt8*^{DTR} mice (177). Injection of DT in Bas-TRECK mice also causes efficient ($\geq$90%) depletion of basophils (174). In this model, the human DTR was inserted under control of the 3' proximal enhancer in the *IL4* locus.

Basoph8 (*Mcpt8*$^{IRES-YP-Cre}$) (178), *Mcpt8*-Cre (179) and *P1-Runx1* (180) are three different mouse models showing constitutive depletion ($\sim$90%) of basophils. The *Mcpt8*-Cre model was developed by engineering a bacterial artificial chromosome transgenic mouse that expresses the Cre recombinase under control of the regulatory elements of *Mcpt8* (179). *Mcpt8*-Cre mice are constitutively deficient for basophils; therefore, this model is suitable for experiments that need long-term ablation of these cells. In the Basoph8 (*Mcpt8*$^{IRES-YP-Cre}$) mice an IRES-YFP-Cre cassette was inserted before the start codon of the *Mcpt8* gene (178). The dysruption of the distal (P1) promoter of the transcription factor Runx1 resulted in >90% depletion of basophils indicating that Runx1 plays a critical role in the development of mouse basophils (180). *Runx1*$^{P1N/P1N}$ mice have markedly reduced numbers of basophils in bone marrow, spleen and peripheral blood (180). Recently, a new mouse model (*Mcpt8*$^{iCre/+}$*Il4*$^{fl/fl}$) was established by crossing two mouse stains, *Mcpt8*$^{iCre/+}$ and *Il4*$^{fl/fl}$ mice (74). These mice are selectively deficient for IL-4 only in basophils and are thus suitable to assess the role of basophil-derived IL-4 in different pathophysiological conditions, including cancer. Several excellent reviews have analyzed in details the different mouse models to investigate basophil functions *in vivo* (75, 119, 181, 182).

It is important to emphasize that previous studies using antibody-depleted basophils (114) and genetically engineered models (62, 91) provided contrasting results on the role of basophils in cancer. Moreover, it should be pointed out that even new mouse mutants have some hematological abnormalities (177). Therefore, results obtained with basophil-deficient mouse models should be interpreted with caution.

PERIPHERAL BLOOD BASOPHILS AND HUMAN CANCER

It has been well-known for some time that basophilia can occur during the advanced phase of chronic myeloid leukemia (CML) (183). The transcription factor IKAROS is markedly reduced in bone marrow from CML patients (184). Overexpression of the dominant-negative isoform of IKAROS in CD34^{+} cells from CML patients resulted in inhibition of IKAROS activity and increased differentiation into basophils (184). Basophils from CML patients express HGF, which promotes CML cell expansion in an autocrine fashion (106). In a mouse model of CML it has been shown that basophil-like leukemia cells promote CML development by producing the chemokine CCL3 (185). In this model basophil-derived CCL3 negatively regulates the proliferation of normal hematopoietic stem/progenitor cells and promotes the expansion of leukemia cells (186). There is also evidence that basophilia is an independent risk factor for evolution of myelodysplastic syndrome to acute myeloid leukemia (187, 188).

Peripheral blood basophils have also been associated with certain solid tumors (189). Basopenia appears to be associated with poor prognosis of colorectal cancer (190, 191), whereas circulating basophils have no predictive role in breast cancer (192), ovarian cancer (54) and oral squamous cell carcinoma (193). Of note, high relative circulating basophils positively associated with improved outcome in melanoma patients undergoing immunotherapy with nivolumab plus ipilimumab (194). On the other hand, baseline basophil count may predict recurrence in patients with high-grade bladder cancer receiving bacillus Calmette-Guérin (BCG) following resection (195). Finally, in a mouse model of breast cancer, a low percentage of circulating basophils correlated with an increased number of pulmonary metastases, suggesting a protective role of basophils in this model (196).

Basophils and Polycythemia Vera

Polycythemia vera (PV) is a myeloproliferative neoplasm characterized by clonal stem cell proliferation of erythroid, megakaryocytic, and myeloid cell lines (197, 198). An activating Janus kinase 2 (JAK2) mutation (JAK2^{V617F} or exon 12 mutation), leading to an overactive JAK-STAT signaling pathway is found in more than 90% of PV patients (199, 200). Pruritus is a common symptom in PV patients (198, 201) and basophil-derived mediators have been implicated in this disorder (202). Absolute basophil counts have been found increased in JAK2^{V617F}-positive PV patients compared to control subjects (203). The expression of CD63, a surface marker of basophil activation, is increased in PV patients with pruritus compared to controls. Finally, PV basophils are hyperresponsive to IL-3 compared to basophils from normal donors. Collectively, these findings indicate that JAK2^{V617F} mutation is associated with hyperreactivity of PV basophils. The latter observation is likely responsible for pruritus in PV patients. Given the role of basophils as major source of Th2 cytokines (e.g., IL-4), we cannot exclude the possibility that the hyperresponsiveness of these cells might play a role in the possible evolution of PV patients.

Basophils and Ovarian Cancer

In a recent study, Bax and co-workers examined the role of basophils in ovarian cancer patients (204). They found that higher percentage of circulating basophils from ovarian cancer patients was positively associated with improved overall survival. Furthermore, by protein and gene expression analyses they detected resting (CCR3, CD123, FcεRI) and activated basophils (CD63, CD203c) in ovarian tumors. Whereas, gene expression for tumor-resident basophils was not associated with patient survival outcomes, gene signatures for activated basophils were positively associated with improved progression-free and overall survival. This study suggests that activated basophils, either in circulation or in tumor, are associated with a survival benefit in ovarian cancer patients.

BASOPHILS AND LUNG CANCER

It has been well-known for some time that murine (62, 73, 124, 125) and human (127–131) basophils have a propensity to migrate into the site of inflammation, including the lung. Whether this influx contributes to the supply of tissue resident basophils that promote M2 polarization of lung macrophages (44) remains to be determined. Nonetheless, the evidence that lung-resident basophils acquire the expression of several cytokines due to the exposure to lung-specific signals (e.g., IL-33, GM-CSF), emphasizes the plasticity of these cells. Thus, basophils migrating into tissue may take on completely new roles, based on the cytokine environment they encounter. The observation that the pulmonary microenvironment may condition the transcriptional and functional development of immune cells has recently been extended to the oncological context. Single-cell transcriptomics of human and mouse lung cancers revealed that blood and tumor neutrophils and monocytes strongly differed in their gene expression (205). Interestingly, basophils were present in mouse lung tumors. Lavin and collaborators compared the simultaneous single-cell analysis of the immune compartments in early (stage I) lung adenocarcinoma, non-involved lung tissue (nLung), and peripheral blood of each patient (50). Basophils were present in both solid tumor site and nLung. A percentage of basophils in the tumor were PD-L1$^+$. This study demonstrates that, as early as in stage I disease, basophils are present in the immune landscape of nLung adenocarcinoma.

In a related example of how the TME can influence basophil function, Schroeder and collaborators demonstrated that highly purified human basophils release histamine and produce IL-4 and IL-13 when co-cultured with the lung carcinoma cell line, A549 (30). Remarkably, these responses required that basophils express IgE, yet occurred independently of allergen, and were suppressed pharmacologically by inhibitors of FcεRI signaling. It was subsequently determined that the IgE-binding lectin, galectin-3, expressed on the A549 cells, was responsible for basophil activation (206). In support of these findings, basophils co-cultured with microspheres coated with galectin-3 also secreted IL-4 and IL-13. Galectin-3 is implicated as a biomarker and/or factor contributing to the pathogenesis of a wide range of conditions, including cancer, cardiovascular disease, autoimmunity, wound healing, and chronic inflammation in general (207). Overall, these findings illustrate a novel mechanism by which galectin-3 expressed by human lung carcinoma cells can activate basophils (and likely other cell types) to release several immunoregulatory cytokines and proinflammatory mediators. Additional studies are required to elucidate the exact role of galectin-3 in activating basophils, and how the mediators and cytokines released by these cells contribute to human and experimental lung cancer.

BASOPHILS AND MELANOMA

The role of basophils has been evaluated in a mouse model of melanoma in which Treg depletion was induced (114). Treg depletion in Foxp3^{DTR} mice was associated with tumor infiltration of basophils and CD8$^+$ T cells leading to rejection of melanoma. Basophils promoted CD8$^+$ lymphocyte infiltration into the tumor through the production of CCL3 and CCL4. Depletion of basophils, through administration of MAR1 (i.e., anti-FcεRI), in Foxp3^{DTR} melanoma-bearing mice prevented the rejection of melanoma, suggesting a pivotal role of basophils in this model. However, as previously mentioned, MAR1 can also deplete/activate other immune cells (e.g., mast cells, DCs, monocytes) expressing FcεRI (170, 171). Thus, the possible role of basophils in melanoma will need to be confirmed using the newer genetically engineered basophil-deficient mouse models.

We recently explored the anti-tumor activity of IL-33, a cytokine known to induce tumoricidal functions in eosinophils (208, 209) on bone marrow-derived murine basophils. Incubation of basophils with IL-33 upregulated granzyme B mRNA and the surface expression of CD63 (80), indicating phenotypic and functional activation. When IL-33-activated basophils were co-cultured with metastatic B16-F10 melanoma cells, tumor cell-growth was substantially inhibited, as compared to melanoma cells co-cultured with resting basophils. These preliminary findings suggest that, under appropriate stimulation, basophils can acquire tumoricidal properties *in vitro*. Whether similar activity occurs *in vivo* remains to be determined, but it is an area of ongoing investigation.

BASOPHILS AND PANCREATIC CANCER

In the mid 1990s, Ann M. Dvorak showed ultrastructural features of piecemeal degranulation of human basophils in the stroma of pancreatic cancer (11). More recently, Protti and collaborators elegantly investigated the role of basophils and their mediators in experimental and human pancreatic cancer (91). In a large cohort of pancreatic ductal adenocarcinoma (PDAC) patients, they found basophils expressing *IL4* in tumor-draining lymph nodes (TDLNs) of PDAC. Importantly, the presence of basophils in TDLNs was an independent negative prognostic biomarker of patient survival after surgery. The authors also examined the possible role of basophils in an orthotopic model of pancreatic cancer using the *Mcpt8*-Cre basophil deficient (179) and WT mice. At 8 weeks after implant, tumor was found in 80% WT, but in none of basophil-deficient mice. The authors demonstrated

the presence of basophils in TDLNs in this model of pancreatic cancer and provided evidence that cancer-associated fibroblasts (CAFs) released TSLP which activated DCs to produce IL-3 from $CD4^+$ T cells. IL-3-activated basophils produced substantial amounts of IL-4. It was further determined that DCs and $CD14^+$ monocytes produced CCL7 which was responsible for basophil migration into TDLNs. Based on these findings, schematically illustrated in **Figure 2**, the authors concluded that basophils can favor both Th2 and M2 polarization through the production of IL-4, thus playing a relevant pro-tumorigenic role in PDAC progression. Consistent with this latter concept of IL-4 driving M2 development, our own *in vitro* studies point to the importance of basophil-derived IL-4 (and IL-13) in promoting M2-like cells (211).

There is compelling evidence that $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells (Tregs) contribute to maintain immune tolerance in the TME (212, 213) particularly in pancreatic cancer (214). A recent study has shown that Tregs can induce the expression of activation markers (CD69, CD203c, and CD13) and promote the release of several cytokines (IL-4, IL-8, IL-13) from human basophils (90). Tregs induced basophil activation through the release of IL-3. It has been suggested that Tregs might also promote tumor evasion by activating basophils to augment and sustain Th2 responses in TME by secreting IL-3 (215).

IGE, BASOPHILS AND SKIN CANCER

IgE is an ancient and the least abundant circulating immunoglobulin isotype (216). It has been suggested that IgE has evolved to provide protection against helminths (217) and environmental toxins such as venoms (218–220). Moreover, dysregulated IgE responses can cause a variety of allergic

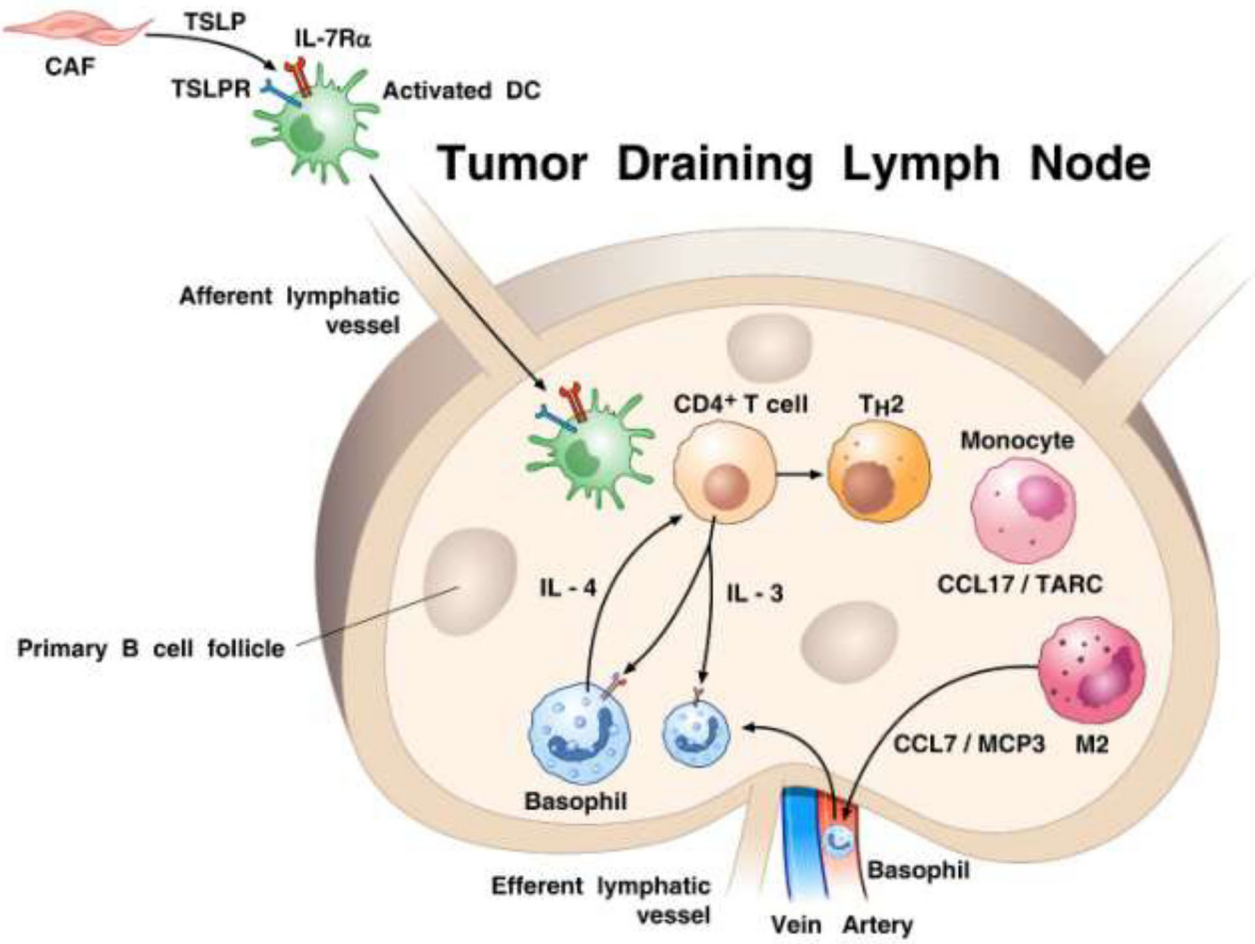

FIGURE 2 | Proposed model of how basophils are recruited and activated in tumor draining lymph nodes (TDLNs) in the context of pancreatic cancer. It has been previously demonstrated (210) that cancer-associated fibroblasts (CAFs) can produce TSLP that engages TSLP receptor on dendritic cells (DCs). TSLP-conditioned DCs migrate into TDLNs were they prime $CD4^+$ T cells for early IL-3 production. Monocytes, which are driven to differentiate toward a M2-type by activated CAFs, release the basophil chemoattractant CCL7/MCP3 (52). Basophils are recruited from afferent arterial blood into lymph nodes and are activated by IL-3 to express IL-4. Basophils are a major source of IL-4 contributing to both Th2 and M2 polarization. The percentage of basophils in TDLNs is an independent negative prognostic factor of survival after surgery of pancreatic cancer patients (91).

disorders (221, 222). IgE binds with very high affinity to FcεRI on mast cells and basophils and remains bound to its receptor for the life of these cells (223). It has been demonstrated that once-weekly topical application of the carcinogen 7,12-dimethylbenz [a] anthracene (DMBA) to the skin of WT mice led to the development of squamous-cell carcinomas (SCCs) after 8–15 weeks associated with high concentrations of serum IgE and infiltration of IgE-bearing basophils in skin and tumors (224). The same group of investigators extended the previous observation by demonstrating that topical application of the proinflammatory agent 12-0-tetradecanoylphorbol-13-acetate (TPA) (2x a week for 2 weeks) to the skin of WT mice increased serum IgE and IgE-bearing basophils in the skin (62). Using a two-stage inflammation driven model of epithelial carcinogenesis (DMBA and subsequent exposure to TPA) (225), they found that mice lacking IgE (*lgh7*$^{-/-}$) were less susceptible to tumor development compared to WT mice. IgE-bearing basophils (Mcpt8^{+}) accumulated inside skin tumors of WT mice. In this model, IgE-signaling was necessary for activation and histamine release from basophils. Infiltrating tissue basophils showed expression of *Cxcr2, Cxcr4*, and *Ptger2* (CRTH2, the PGD_2 receptor). Blocking CXCR4 with a neutralizing antibody selectively reduced basophil infiltration to the inflamed skin. TSLP and IL-3, abundantly expressed in inflamed skin, increased the surface expression of CXCR4 on basophils, allowing their recruitment to the skin in response to CXCL12. Blocking TSLP and IL-3 simultaneously with neutralizing antibodies abolished basophil recruitment to the skin. The *Mcpt8*$^{Cre/+}$ mice, which have normal mast cell numbers but strongly reduced basophils (179), were less susceptible to tumor growth. Together, these results indicate that in this inflammation-driven model of epithelial carcinogenesis, tumor promotion is mediated *via* FcεRI signaling in skin-infiltrating basophils.

CONCLUSIONS AND OUTSTANDING QUESTIONS

For several decades, basophils were considered erroneously as primary effector cells participating solely in allergic disorders (226, 227). The concept that they might possess immunomodulatory roles became more widely appreciated when murine (5) and human basophils were shown to produce a variety of cytokines (e.g., IL-4, IL-3, and IL-13) (21, 89, 92, 93, 97, 99, 110), which at the time, were thought to be made only by Th2 cells. In addition, there is now compelling evidence that human basophils can synthesize several canonical (57, 86, 105, 106) and non-canonical angiogenic factors (151). It has long been known that human (127–131) and mouse (62, 73, 124–126) basophils have a propensity to migrate from peripheral blood into sites of inflammation. Moreover, basophils were identified in human lung (50), gastric (127, 128), pancreatic (11, 91) and ovarian cancer (204). It was recently shown, at least in mice, that basophils are present in all phases of lung development (44), and display a divergent phenotype from peripheral blood. These resident basophils can favor M2 polarization of lung macrophages, as occurs in several tumors (132, 133). Studies are urgently needed to characterize the presence and the state of activation of basophils in TME and their possible roles in early *vs.* late stages of human and experimental tumors.

Human basophils are a major source of several canonical angiogenic factors such as VEGF-A and VEGF-B (57), HGF (106), ANGPT1 (105), and CXCL8 (86, 89, 90, 228). An elegant study has recently demonstrated that LTC_4 and LTD_4, also produced by human basophils (83), promote tumor angiogenesis and metastasis through the engagement of $CysLT_2R$ on endothelial cells (151). Collectively, these findings suggest that further *in vitro* and *in vivo* investigations should evaluate the roles of canonical and non-canonical angiogenic factors produced by basophils in experimental and human tumors.

Activated human and mouse basophils release BETs (116–118). There is mounting evidence that extracellular DNA traps have multiple effects in cancer (160) favoring tumor growth (167), awakening dormant cancer cells (165), and promoting metastasis in mouse models and in humans (161, 164). Further studies should evaluate the presence of BETs in experimental and human cancers and whether basophil extracellular traps modulate tumor growth and the formation of metastasis *in vivo*.

There are contemporary and developing models/techniques that should greatly facilitate this area of investigation. For example, basophil-deficient mice are powerful models for analyzing basophil functions *in vivo,* but, in some instances, have produced erroneous findings. For example, models using antibody-depleted basophils (168, 169) can often result in the activation of other immune cells (170, 171). Indeed, such models provided highly controversial results on the role of basophils as APCs (95, 170, 172, 173, 229, 230). It is therefore not surprising that basophils may appear to play a protective (114) or a pro-tumorigenic role (62, 91) depending on the experimental model utilized. In general, mouse models with constitutive or inducible basophils depletion should be preferred, but need to take into consideration that even new mouse mutants can have hematologic abnormalities (177) and/or show incomplete removal of basophils. Studies attempting to evaluate basophil functions in a complex and heterogeneous disorder, such as cancer should be performed using multiple genetically engineered models of basophil deficiency.

In conclusion, the last years have witnessed exceptional progress in our understanding of basophil biology. Recent studies have demonstrated that basophils are present in the immune landscape of human (50, 91, 204) and experimental (62, 91) tumors, play a role in lung development and M2 macrophage polarization (44), and participate in canonical (57, 105, 106, 145) and non-canonical angiogenesis (151), and release BETs (117, 118). Further investigations are required before we unravel the mysterious role of basophils in experimental cancer and, more importantly, in humans. The elucidation of basophil role in tumor immunity will require studies of increasing complexity beyond those assessing their microlocalization. High dimensional analysis, particularly single-cell RNA-seq of immune landscape of human and experimental tumors will be of paramount importance in characterizing basophil role in different human and experimental cancers.

AUTHOR CONTRIBUTIONS

All authors contributed to reviewing the current literature and writing of the manuscript.

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Thymic Stromal Lymphopoietin and Cancer: Th2-Dependent and -Independent Mechanisms

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The thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine originally cloned from a murine thymic stromal cell line, and subsequently a human homolog was identified using database search methods. Human TSLP is mostly expressed in epithelial cells, among which are keratinocytes as well as stromal cells such as fibroblasts and immune cells. Human TSLP was first described to activate myeloid dendritic cells, which prime naïve T helper cells to produce high concentrations of Th2 cytokines, thus representing a key cytokine in triggering dendritic cells-mediated allergic Th2 inflammation. TSLP and/or its receptor has been shown to be expressed in several tumor types, where TSLP expression is associated with functional activities that can be associated or not with the induction of a Th2-prone tumor microenvironment, i.e., Th2-dependent and Th2-independent mechanisms. These mechanisms involve tissue- and immune cell target-dependent tumor-promoting or tumor-suppressive functions in different or even the same tumor type. Here we report and discuss the Th2-dependent and Th2-independent roles of TSLP in cancer and possible therapeutic targeting.

Keywords: TSLP, tumor cells, cancer associated fibroblasts, dendritic cells, Th2 inflammation, CD4$^+$ Th2, TSLPR, IL-1

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INTRODUCTION

The thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine originally cloned from a murine thymic stromal cell line (1), and a cDNA clone encoding human TSLP was then identified using database search methods (2, 3). A low affinity TSLP receptor (TSLPR) was isolated (4–7), most closely related to the common γ-chain (6). Subsequently, a functional high-affinity TSLPR complex was defined as a heterodimer formed by the TSLPR and the IL-7 receptor α-chain (5, 6). This receptor combination results in predominant STAT-5 activation and increased cell survival, proliferation, and differentiation to TSLP stimulation (2, 8–10).

TSLP is primarily expressed by epithelial cells at barrier surface, with the highest levels in skin, gut, and lung (11). Expression has been also described in smooth muscle cells and fibroblasts (12). Moreover, dendritic cells (DCs) (13), and possibly other immune cells such as mast cells, can produce TSLP (14). Analysis of the expression profile of TSLPR and IL-7 receptor α-chain subunits showed the highest co-expression of the two receptors in myeloid DCs (3). Several other immune cells from the innate (i.e., macrophages, monocytes, masts cells, neutrophils, eosinophils, NKT cells, and ILC2 cells) and adaptive (i.e., B cells, T cells, Th2 cells, CD8$^+$ T cells, and regulatory T cells [Tregs]) immunity are a cellular target for TSLP, as well as other non-immune cells, such

as platelets and sensory neurons (14, 15). TSLP expression is induced by proinflammatory stimuli, comprising IL-1 (16, 17).

TSLP had been initially implicated in allergic diseases, where it creates a predominant Th2 microenvironment, mostly through DC activation (i.e., upregulation of OX40L, CD80, and CD86) (18), by phosphorylation of several STAT proteins and NFκB (8, 10). More recently, a role for TSLP has been also reported in chronic inflammatory and autoimmune disorders and in cancer (14, 15). We refer readers interested in comprehensive synopses on the role of TSLP in several disease settings to those reviews. Here, we focus exclusively on the literature regarding TSLP expression and function in cancer with special emphasis on the association or not with Th2 inflammation.

TSLP ISOFORMS IN CANCER

Two TSLP isoforms have been identified in human bronchial epithelial cells (19) and are termed long- (i.e., the original one) and short-form TSLP. The short-form TSLP mRNA is constitutively expressed in bronchial and colonic epithelial cells, keratinocytes, and lung fibroblasts (19). Short-form TSLP is believed to exert homeostatic and anti-microbial activities (15, 20), and expression of one or the other or both isoforms in barrier surface diseases have been reported (20).

In cancer the expression of the two isoforms was evaluated in breast (21) and pancreatic (22) cancers. In breast cancer cells both isoforms were upregulated upon stimulation with IL-1β (21). In pancreatic cancer associated fibroblasts (CAFs), variable levels of short-form TSLP mRNA were expressed at the steady state that did not significantly increase upon activation, whereas long-form TSLP mRNA levels significantly increased after activation with proinflammatory cytokines (22), suggesting that the inducible form of TSLP was primarily the long one.

TSLP IN CANCER: HISTORICAL PERSPECTIVE

The first identification of a role for TSLP in cancer was in pancreatic (23) and breast cancers (24, 25), in which TSLP, secreted by either CAFs or tumor cells, respectively, was found to exert tumor-promoting functions through the establishment of predominant Th2-type inflammation in the tumor microenvironment. Previous studies from the same authors reported the presence of carcinoembryonic antigen-specific Th2 cells in the blood of pancreatic cancer patients undergoing surgery that correlated with the presence of predominant GATA-3 positive lymphoid cells in the tumor stroma (26), and of inflammatory IL-13 secreting Th2 cells in primary breast cancer that contributed to accelerate tumor development in a humanized mouse model (27). In addition, in the 4T1 mouse model of breast cancer, an allergic response in the lung was required to favor metastasis formation (28). These data prompted the authors to look for mechanisms leading to Th2 inflammation in these tumors, and they hypothesized that, due to its function in Th2 allergic responses, TSLP could be a relevant candidate to investigate.

Following the first reports in pancreatic and breast cancer, several studies also in other tumors found either pro-tumor or anti-tumor activity of TSLP, and through Th2-dependent as well as Th2-independent mechanisms. This distinction is mostly based on the association between TSLP expression and the development of predominant Th2 inflammation in the tumor or direct TSLP signaling on TSLPR expressing tumor cells. These studies are summarized in **Table 1**.

Th2-DEPENDENT MECHANISMS OF TSLP IN CANCER

Chronic inflammation is associated with tumor development and progression (50, 51). While Th1-dependent acute inflammation has been associated with tumor rejection, Th2-dependent chronic inflammation is believed to enable tumor growth (52, 53). As mentioned above, TSLP promotes predominant Th2-type inflammation in different tumors and mediates pro-tumor but also anti-tumor functions (**Table 1**). In order to exert its Th2 polarizing effects, TSLP can either indirectly act through myeloid DC conditioning that supports Th2 cell priming/differentiation from naïve CD4 T cells (18) or directly bind to $CD4^+$ T cells, which upregulate the TSLPR upon activation (54, 55), with higher expression on Th2 compared with Th1 and Th17 cells (9), suggesting that direct TSLP-TSLPR signaling occurs in antigen-specific memory T cells.

Th2-dependent mechanisms of TSLP in cancer have been reported in pancreatic, breast, skin, gastric, and oropharyngeal cancers, with pro- and anti-tumor effects, as detailed below.

Pancreatic Cancer

A tumor-promoting function for TSLP was demonstrated in pancreatic cancer, where predominant Th2 ($GATA\text{-}3^+$) over Th1 ($T\text{-}bet^+$) cells within the lymphoid infiltrate in the tumor stroma was associated with reduced survival in pancreatic cancer patients, thus implying an active role for Th2 immunity in tumor progression (23). TSLP expression in the tumor was significantly higher than in the surrounding tissue, and, as reported above, it was supported by CAFs activated by tumor-derived cytokines. *In vitro* studies demonstrated that DCs activated with the supernatant of activated CAFs induced TSLP-dependent Th2 cell polarization of naïve $CD4^+$ T cells (**Figure 1B**). Importantly, *in vivo* TSLPR expressing DCs were present in the tumor stroma and in tumor-draining but not in non-draining lymph nodes (LNs). The following studies identified a complex crosstalk in the tumor microenvironment and tumor-draining LNs (TDLNs) relevant to the establishment of TSLP-dependent Th2-type inflammation in pancreatic cancer. The authors reported that tumor-derived IL-1, released by tumor cells and inflammasome adaptor ASC-activated M2 cells, is crucial for TSLP secretion by CAFs (22) (**Figure 1A**), and that IL-4 derived by basophils, recruited into TDLNs by alternatively

Abbreviations: CAF, cancer associated fibroblasts; CTCL, cutaneous T cell lymphoma; DC, dendritic cell; LN, lymph node; TDLNs; tumor-draining LNs; Tregs, regulatory T cells; TSLP, thymic stromal lymphopoietin; TSLPR, TSLP receptor; WT, wild-type.

TABLE 1 | TSLP expression and pro-tumor or anti-tumor function in human and mouse cancers.

Tumor type	TSLP expression	TSLP function	Human/ Mousemodels	Th2-dependent mechanisms	Th2-independent mechanisms	Clinical correlates	References
Pancreatic cancer	CAFs	Pro-tumor	Human	CAF-derived TSLP activates myeloid DCs with Th2 polarizing capability (IL-13 producing CD4 T cells). $CD11c^+TSLPR^+$ cells are present in the tumor and tumor draining LNs		$GATA\text{-}3^+/T\text{-}bet^+$ cells ratio is an independent predictive factor of survival after surgery in pancreatic cancer patients	(23)
Pancreatic cancer	Skin keratinocytes, systemic	Anti-tumor	KPC cells (29) subcutaneously implanted in K14-$TSLP^{tg}$ mice	GATA-3 cells in the tumor of K14-$TSLP^{tg}$ are significantly increased compared to WT mice		Tumors in K14-$TSLP^{tg}$ grow less than in WT mice	(30)
Breast cancer	Tumor cells	Pro-tumor	Human and humanized NOD/SCID/β2m KO mice subcutaneously implanted with human breast cancer cells	Tumor cell-derived TSLP activates myeloid DCs with Th2 polarizing capability (IL-13 and TNFα producing CD4 T cells). $CD11c^+OX40L^+$ are present in the tumor		Anti-OX40L and anti-TSLP antibodies significantly prolong survival in humanized immune-deficient mice	(25)
Breast cancer	4T1 tumor cells	Pro-tumor	4T1 cells subcutaneously implanted in TSLPR KO mice	TSLP-TSLPR signaling in Th2 cells		4T1 cells grow significantly less in TSLPR KO mice and give less metastases in the lung	(24)
Breast cancer	4T1 tumor cells	Pro-tumor	4T1 cells subcutaneously implanted in TSLPR KO mice	Immune response is shifted toward Th1 in TSLPR KO mice		4T1 cells grow significantly less in TSLPR KO mice and give less metastases in the lung but more in the brain	(31)
Breast cancer	Skin, systemic	Anti-tumor	K14-$TSLP^{tg}PYMt^{tg}$ mice $PYMt^{tg}$ cells implanted in TSLPR KO	GATA-3 cells in the tumor of K14-$TSLP^{tg}PYMt^{tg}$ are significantly increased compared to single transgenic $PYMt^{tg}$ mice		Tumor lesion numbers are significantly lower in double transgenic mice	(30)
Breast cancer	Myeloid cells	Pro-tumor	Orthotopic implant of TSLP- and TSLPR-deficient 4T1 cells MMTV-PyMT mice		Tumor-derived IL-1α induces TSLP expression in myeloid cells that in turn activated anti-apoptotic pathways in $TSLPR^+$ tumor cells TSLP expression in lung is necessary for metastases	TSLP deficient mice implanted with 4T1 cells have smaller primary tumors and fewer lung metastases than WT mice Lung metastases are reduced by anti-TSLP antibody	(32)
Breast cancer	4T1 and KCMH-1 cells	Pro-tumor	4T1 orthotopic implantation in syngeneic mice		Tumor-derived TSLP induces the expression of tissue remodeling and angiogenic genes in alveolar macrophages	Reduced lung metastases in TSLP-KO bearing mice	(33)
Breast cancer	4T1 cells	Pro-tumor	4T1 orthotopic implantation in syngeneic mice TSLPR KO mice		TSLP promotes pre-B cell emigration from the bone marrow, and their survival/expansion in the periphery. Tumor cells favor conversion of pre-B cells into regulatory B cells that affect antitumor immunity and favor lung metastases	Reduced lung metastases in TSLP-KO bearing mice	(34)
Lung cancer	Tumor cells	Pro-tumor	Human		TSLP-conditioned DCs induce Tregs TSLP expression in the tumor correlates with the number of $FoxP3^+$ Tregs	TSLP expression correlates with pathologic type, stage, tumor size, and LN metastases	(35)

(Continued)

TABLE 1 | Continued

Tumor type	TSLP expression	TSLP function	Human/ Mousemodels	Th2-dependent mechanisms	Th2-independent mechanisms	Clinical correlates	References
Cervical cancer	Tumor cells	Pro-tumor	Human		Tumor-derived TSLP induces recruitment and proliferation of eosinophils that in turn promote tumor cell proliferation and inhibit apoptosis		(36)
Cervical cancer	Tumor cells	Pro-tumor	Human		TSLP promotes angiogenesis through eosinophil-derived factors		(37)
Cervical cancer	Tumor cells	Pro-tumor	Human		TSLP promotes tumor cell proliferation and invasion		(38)
Skin cancer	Keratinocytes	Anti-tumor	Mice KO in Notch signaling CD1 mice treated with DBA and TPA	Tumors in KO compared to WT mice are infiltrated by an higher percentage of Th2		Blocking TSLP signaling induces skin tumorigenesis	(39)
Skin cancer	Keratinocytes	Anti-tumor	Notch and/or β-catenin mutant mice crossed with TSLPR KO mice Mice constitutively expressing β-catenin and TSLPR KO		TSLP-TSPR signaling increased CD8 T cell fitness and reduced CD11b+Gr1+ cells	TSLP-TLSPR signaling protects against tumor formation	(40)
Skin cancer	Keratinocytes	Anti-tumor	Barrier protein deficient mice (EPI-/-) treated with DMBA and TPA		TSLP and NKG2D restrained skin carcinogenesis		(41)
Cutaneous T-cell lymphoma	Keratinocytes	Pro-tumor	Human and EL-4 and MBL-2 cell model	TSLP induces IL-4 and IL-13 expression by tumor cells through STAT5 activation	TSLP signaling induces proliferation of TSLPR+ tumor cells	Anti-TSLP antibody in mouse models reduces tumor formation	(42)
Colorectal cancer	Tumor cells	Anti-tumor	Human and xenograft model (subcutaneous injection of human tumor cells in nude mice)		TSLP-TSLPR signaling induces apoptosis	TSLP administration in mouse models inhibits colon tumor growth	(43)
Gastric cancer	Tumor cells	Pro-tumor	Human			TSLP overexpression correlates with LN metastases	(44)
Gastric cancer	Tumor cells	Pro-tumor	Human	Previous report (45) showed that infection by H. pylori induces release of TSLP from gastric cells that in turn trigger a Th2 response through DC activation		Prognosis in patients with $TSLP^+$ tumor is worse than in patients with $TSLP^-$ tumors TSLP serum levels are independent prognostic indicators	(46)
Ovarian carcinoma	Tumor cells	Pro-tumor	Human			TSLP is an independent predictive factor of reduced survival	(47)
Oropharyngeal squamous cell carcinoma	Tumor cells	Pro-tumor	Human	High IFNγ, and low IL-4, TSLP, and TGF-β correlates with increased survival		Low TSLP expression is a good prognostic factor	(48)
B cell precursor acute lymphoblastic leukemia	Not reported	Pro-tumor			TSLP-TSLPR signaling induces tumor cell proliferation and signal transduction		(49)

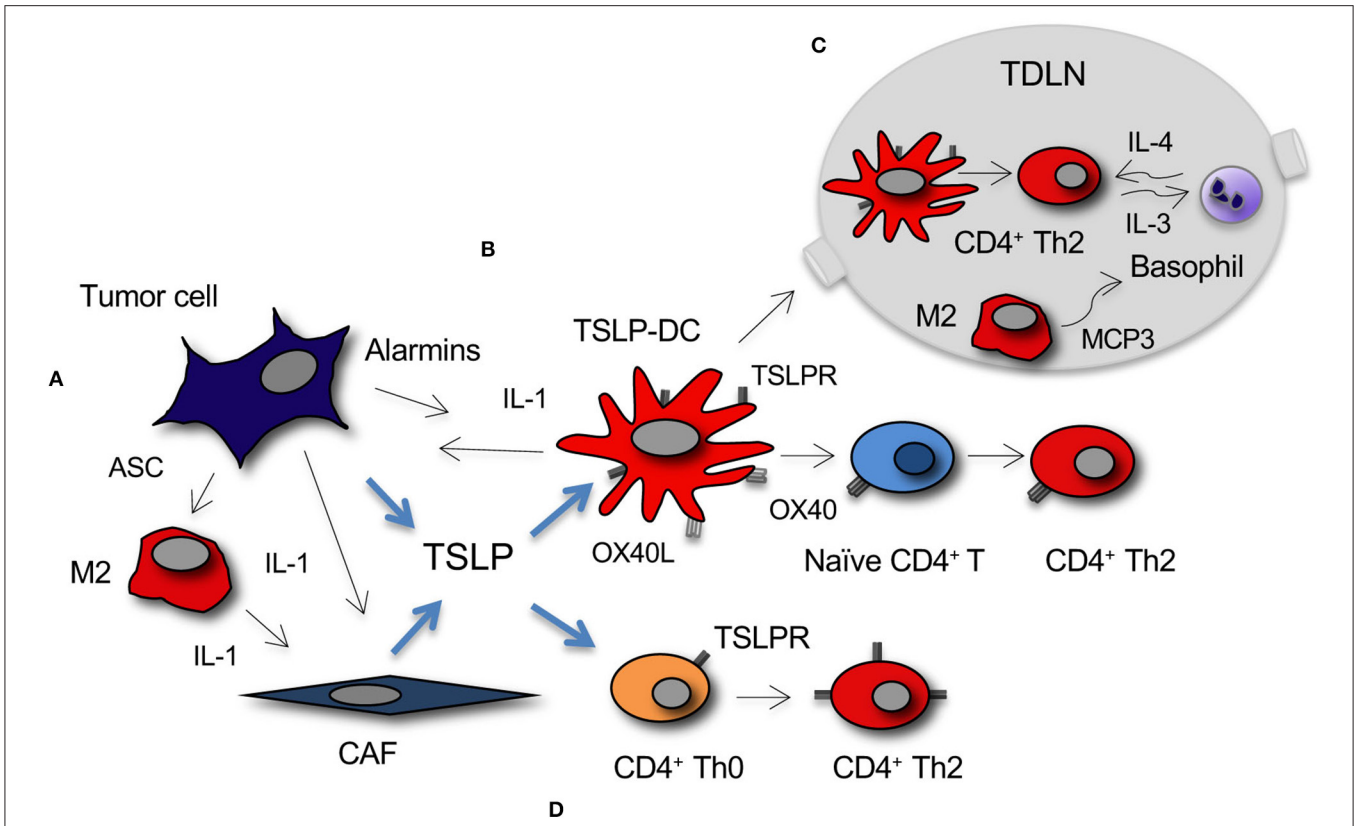

FIGURE 1 | Development of TSLP-dependent protumor Th2 inflammation in cancer. **(A)** TSLP secretion by tumor cells and CAFs is primarily induced by tumor-derived IL-1. IL-1 is released directly from tumor cells or myeloid DCs and M2 macrophages within the stroma under the influence of tumor cell-derived alarmins, among which the inflammasome adaptor ASC. **(B)** Myeloid DC conditioned by TSLP (TSLP-DC) prime and polarize naïve $CD4^+$ T cells toward a Th2 phenotype through TSLP-TSLPR and OX40-OX40L signaling. **(C)** TSLP-DC migrate to TDLNs where they prime $CD4^+$ Th2 cells that secrete IL-3. M2 macrophages present in TDLNs secrete MCP3 that recruit basophils, which after activation by Th2-derived IL-3, secrete the IL-4 required for GATA-3 stabilization in $CD4^+$ Th2 cells. **(D)** TSLP directly induces $CD4^+$ T cell polarization toward Th2 and/or expansion of Th2 effectors through TSLP-TSLPR signaling.

activated M2 macrophages, stabilizes the Th2 polarization (56) (**Figure 1C**), thus adding further complexity to the crosstalk within the tumor microenvironment that leads to predominant Th2 inflammation in pancreatic cancer (57). M2 macrophages and $CD4^+$ Th2 present in the tumor microenvironment possibly mediate tumor progression by favoring invasion and metastasis formation, as it has been shown in a breast cancer model (58).

In contrast with the data reported in the human disease, tumor growth was reduced in a transplantable mouse model of pancreatic cancer, where transgenic mice overexpressing TSLP in the skin (K14-TSLPtg) were subcutaneously injected with syngenic pancreatic cancer cells, compared to the wild-type (WT) controls (30). Tumors from these transgenic mice had increased numbers of infiltrating $CD4^+$ Th2 cells compared to WT mice, suggesting that in this model TSLP and Th2 cells exerted tumor-suppressive function in the context of a systemic Th2-polarized environment.

At difference with the transplantable K14-TSLPtg mouse model reported above (30), very recently DePinho and collaborators (59), using a transgenic mouse model of pancreatic cancer carrying an inducible oncogenic KRAS mutation, demonstrated a tumor-promoting function for Th2 cytokines from the tumor microenvironment, thus recapitulating the human disease. In this model, activation of cancer cells carrying the mutated KRAS by IL-4 and IL-13, which were secreted by the Th2 cells present in the tumor microenvironment, triggered the JAK1-STAT6-MYC pathway that in turn activated glycolysis crucial for tumor progression.

Breast Cancer

Concomitantly and similarly to human pancreatic cancer, a tumor-promoting role for TSLP was demonstrated in breast cancer (25). The authors showed that human breast cancer cells directly produce TSLP, and that tumor cell derived-TSLP induces *in vitro* OX40L expression on DCs (25) (**Figure 1B**). OX40L-expressing DCs were found in primary breast tumor infiltrates and *in vitro* they drove the development of inflammatory Th2 cells (i.e., producing IL-13 and TNF-α). Importantly, in a xenograft mouse model, anti-TSLP or anti-OX40L neutralizing antibodies inhibited breast tumor growth and IL-13 production. Studies from the same group (21) showed that, similarly to pancreatic cancer (22), IL-1β, which was released by myeloid DCs

under the influence of tumor-derived factors (i.e., alarmins), was key for TSLP secretion by breast cancer cells (**Figure 1A**).

A role for TSLP in breast cancer progression and metastasis to the lungs was also reported in Olkhanud et al. (24). In the 4T1 orthotopic murine model TSLP was produced by cancer cells that directly acted on TSLPR-expressing $CD4^+$ T cells to induce their Th2 differentiation (**Figure 1D**). TSLP was also expressed by human breast metastases in the lung, and in the murine model the metastatic potential of different 4T1 cell clones was associated with their ability to secrete TSLP. *In vivo* TSLP functional inactivation either by silencing or by using TSLPR KO mice demonstrated the role of tumor-derived TSLP in inducing a metastases prone environment in the lungs. This was due to secretion by $CD4^+$ T cells of pro-tumor Th2 cytokines (i.e., mainly IL-10 and IL-13), possibly with activation of $CD4^+$ NKT and myeloid suppressive cells (60, 61), and tumor-derived CCL17 that in turn recruited Tregs already described to have a pro-metastatic role in breast cancer (28).

The function of TSLP in favoring development of primary breast cancer and lung metastasis was subsequently confirmed in the same 4T1 transplantable model where cancer cells were grown in TSLPR KO mice (31). Lack of functional TSLPR mitigated Th2 polarization as well as the establishment and growth of primary breast tumors and lung metastases. Unexpectedly, in the same model brain metastases were found enhanced, suggesting a protective role for TSLP in this site.

In contrast with the results discussed above, Demehri et al. (30) found a tumor-suppressive role for TSLP in murine models of breast carcinogenesis. In order to determine the impact of systemic TSLP on the early stages of breast cancer development, the authors used two murine models. In one model they crossed the MMTV-polyoma middle T ($PyMt^{tg}$) breast cancer-prone with the K14-TSLPtg mice (K14-$TSLP^{tg}PYMt^{tg}$), whereas in the other model WT mice were topically treated with calcipotriol, which is known to induce TSLP expression in mouse keratinocytes (62). In both experimental settings breast cancer cells were exposed to high levels of circulating TSLP, were arrested at an early adenoma-like stage, and were prevented from advancing to late carcinoma and metastases. In both models $CD4^+$ Th2 cells were shown to mediate the tumor-suppressive effects of TSLP.

A further level of discussion on the pro- vs. anti-tumor role for TSLP in breast cancer was shared by Soumelis and collaborators (63), who did not find TSLP expression in the majority of human tumor samples examined as well as TSLPR expression in tumor infiltrating immune or stromal cells, suggesting lack of TSLP-TSLPR signaling in breast cancer.

Skin Cancer

Conflicting results were also reported in skin cancer. Demehri et al. (39) reported a tumor-suppressive role for TSLP in skin carcinogenesis by using mice with clonal loss of Notch signaling in their skin. In this model, high levels of TSLP released by barrier-defective skin caused severe inflammation that resulted in gradual elimination of Notch-deficient epidermal clones and resistance to skin carcinogenesis. Overexpression of TSLP in WT skin by chemical induction with calcipotriol also caused resistance to tumorigenesis. As in the breast cancer models reported above, $CD4^+$ Th2 cells mediated the tumor-suppressive effect of TSLP in these models of skin carcinogenesis.

In contrast, Takahashi et al. (42) reported that cutaneous T cell lymphoma (CTCL) lesions in advanced stages exhibited a Th2-dominant phenotype. *In vitro* CTCL cell lines and peripheral blood mononuclear cells from Sezary syndrome patients showed increased IL-4 and IL-13 expression in response to TSLP, through the activation of STAT5.

Gastric Cancer

In gastric cancer patients TSLP expression in the tumor correlated with worse prognosis, and high serum concentration of TSLP was identified as an independent prognostic factor of reduced survival (46). A previous study from the same group (45) had shown that *Helicobacter pylori* infection induced gastric epithelial cells to secrete inflammatory cytokines, among which are TSLP. In addition, *in vitro* DCs conditioned by the supernatant of Helicobacter-infected epithelial cells triggered differentiation of T cells with a mixed Th1/Th2 profile, and TSLP was found to be responsible for the Th2 cytokine production.

Oropharyngeal Squamous Cell Carcinoma

Finally, analyses of surgical specimens of oropharyngeal squamous cell carcinoma indicated that high IFN-γ and low IL-4, low TSLP, and low TGFβ expression was associated with better prognosis in oropharyngeal squamous cell carcinoma patients (48).

Collectively, in the majority of studies TSLP and Th2 inflammation exerted pro-tumor activity. Conflicting results were reported in pancreatic, breast, and skin cancers.

Th2-INDEPENDENT MECHANISMS OF TSLP IN CANCER

In the majority of models Th2-independent mechanisms of TSLP in cancer rely on direct TSLP-TSLPR signaling in TSLPR-expressing tumor cells involving apoptotic pathways, tumor cell proliferation, signal transduction, and activation of remodeling and proangiogenic gene signatures (**Table 1**).

Th2-independent mechanisms of TSLP in cancer have been reported in breast, lung, cervical, skin, and blood cancers, with pro- and anti-tumor effects, as detailed below.

Breast Cancer

In breast cancer, three studies (32–34) demonstrated a tumor-promoting role for TSLP. In one study (32), TSLP produced by myeloid cells after activation with tumor cell-derived IL-1α activated anti-apoptotic pathways in TSLPR-expressing tumor cells, through Bcl-2. Experiments in TSLP KO mice then showed that TSLP signaling was required for metastatic disease progression to the lung. In another study (33), tumor cell-derived TSLP induced invasive and angiogenic gene expression profiles in alveolar macrophages. Depletion of alveolar macrophages but not macrophages from the circulation impacted lung lesion growth. A role for TSLP in driving lung metastases was also recently reported in Ragonnaud et al. (34), where tumor cell-derived TSLP

induced pre-B cell emigration from the bone marrow through CXCR4 and α4β1 downregulation and promoted their survival and expansion. These pre-B cells were induced by tumor cells to differentiate into regulatory B cells that in turn downmodulated anti-tumor immunity and promoted lung metastases.

Lung Cancer

A tumor-promoting function for TSLP was described in lung cancer (35), where TSLP expression in the tumor tissue was higher compared to the normal counterpart. *In vitro* experiments showed STAT-1,−3, and−5 phosphorylation in TSLP-DCs that favored recruitment and differentiation of Tregs, possibly through CCL22 and TGFβ secretion, respectively, and in lung cancer patients the prevalence of Tregs correlated with TSLP expression in the tumor.

Cervical Cancer

Several studies reported a pro-tumor role for TSLP in cervical cancer (36–38). Tumor cells under hypoxia expressed TSLP, and TSLPR was expressed in both tumor cells and vascular endothelial cells. TSLP caused the release of CCL17 by tumor cells with recruitment of eosinophils that in turn induced proliferation and restricted tumor cell apoptosis through up-regulation of Ki-67 and Bcl-2, respectively (36) and of proangiogenic factors (37). TSLP also promoted proliferation and invasion of cervical cancer cells by downregulating microRNA-132 (38).

Gastric and Ovarian Cancer, and B Cell Precursor-Acute Lymphoblastic Leukemia

A pro-tumor activity for TSLP was described in gastric (44) and ovarian (47) cancer patients where TSLP overexpression in tumor compared to normal tissue correlated with LN metastases (44), and TSLP expression was identified as an independent predictive factor of reduced survival (47). In addition, Vetter and collaborators (49) showed that in a fraction (about 20%) of patients with B cell precursor-acute lymphoblastic leukemia tumor cells expressed the TSLPR, and *in vitro* stimulation of leukemic cells with TSLP enhanced their proliferation and induced activation of STAT-5 signaling.

Skin Cancer

Conflicting results were instead obtained in skin cancer, where TSLP production by keratinocytes was associated with both pro-tumor and anti-tumor activity. In CTCL, fibroblast-derived periostin mediated TSLP production by keratinocytes that in turn directly stimulated *in vitro* tumor cell growth in TSLPR-expressing tumor cells, and *in vivo* TSLP inhibition reduced tumor formation in EL-4 and MBL-2 cell mouse models (42). A Th2-dependent tumor-promoting role for TSLP in CTCL was also described (see above). On the contrary, in another study Di Piazza et al. (40), using several transgenic and knockout mouse models, demonstrated that TSLP prevented skin carcinogenesis. This effect was mediated mainly by $CD8^+$ T cells, possibly because TSLP-TSLPR signaling increased their survival/proliferation. In addition, ablation of the TSLP-TSLPR signaling induced recruitment and/or development of $CD11b^+Gr1^+$ cells that was dependent on epithelial-specific Wnt/β-catenin signaling. These cells directly promoted tumor growth by increased provision of Wnt ligands and not indirectly by acting on T cells. In partial agreement with the report of Di Piazza et al. (40), Cipolat et al. (41) showed that barrier proteins KO (EPI-/-) mice are highly resistant to developing tumors when treated with DMBA and TPA. TPA induced an exaggerated atopic response, immune infiltration, and elevated levels of circulating TSLP. This could be normalized by blocking TSLP or NKG2D but not $CD4^+$ T cells. However, it is difficult to explain why mice with lesions $>$ 2 mm had higher levels of TSLP compared with those with lesions $<$ to 2 mm.

Colorectal Cancer

Finally, an anti-tumor role for TSLP was reported in colorectal cancer (43), where its expression in the tumor was significantly lower than in surrounding tissues, and negatively correlated with clinical staging in colorectal cancer patients. At difference with the anti-apoptotic function reported (2), in this model TSLP enhanced *in vitro* tumor cell apoptosis through caspase-3,−8, and−9 activation, and TSLP administration in xenograft models reduced tumor growth.

Collectively, in the majority of studies through Th2-independent mechanisms, TSLP exerted pro-tumor activity (i.e., breast, lung, cervical, and blood cancers). Conflicting results were reported in skin cancer.

CONCLUSION

In the past decade a role for TSLP has been clearly identified in several cancers with somewhat conflicting results, depending on the tumor but even within the same tumor type. In human studies TSLP expression was always associated with a pro-tumor function with the exception of colorectal cancer, whereas an anti-tumor function was found in those mouse models (i.e., pancreatic, breast, and skin cancers), in which high levels of systemic TSLP were reached (**Table 1**). These data possibly suggest that, independently of the tumor type, also the local vs. the systemic expression of TSLP highly affects its final functional outcomes.

In the majority of studies TSLP-dependent Th2 inflammation was associated with tumor-promoting functions; however, in mouse models of breast and skin carcinogenesis, Th2 cell polarization was associated with tumor-suppressive functions. Possible explanations for these discrepancies can be envisaged. In breast (30) and skin carcinogenesis (39), where transgenic mice express TSLP in their skin keratinocytes, high levels of systemic TSLP were also present (see above), suggesting a possible generalized skew in Th2-type immune responses. Indeed, in these models Th2 cell responses are the only possibly induced tumor-elicited immune responses. Another possible and interesting explanation might be related to different phases of disease development (i.e., early vs. more advanced stages). It has been reported (64) that IL-13 derived from intraepithelial lymphocytes regulates tissue homeostasis during skin injuries and protects against skin carcinogenesis. It is tempting to speculate that, especially at barrier sites, Th2 cell

responses might be relevant in early stages carcinogenesis when tissue repair is ongoing. However, when tumors are established, Th2 cells/cytokines become not only insufficient compared to Th1 cells/cytokines as anti-tumor effectors but also promote a chronic tissue repair program, which facilitates the activation of a pro-angiogenic and pro-metastatic tumor microenvironment.

Recently, asthma exacerbations were prevented by an anti-TSLP monoclonal antibody (65), making this therapy also available in tumor types, in which a proven tumor-promoting role of TSLP has been established. In addition, preclinical evidence also suggested the possibility to manipulate the TSLP secretion by modulation of its production. Indeed, IL-1 was shown to be a key factor for activation of TSLP secretion in both pancreatic (22) and breast cancer (21), where the use of the IL-1R antagonist anakinra reduced TSLP availability *in vitro* and in vivo. On the opposite side, treatment with calcipotriol, which increases TSLP levels, in combination with 5-fluorouracil was superior to combination with Vaseline in reducing actinic keratosis lesions (66).

Collectively, whereas a role for TSLP in cancer is firmly established, manipulation of its expression for therapeutic purposes will need further definition of its pro-tumor vs. anti-tumor function in the different tumor types.

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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Anti-Tumorigenic Activities of IL-33: A Mechanistic Insight

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Interleukin-33 (IL-33) is an epithelial-derived cytokine that can be released upon tissue damage, stress, or infection, acting as an alarmin for the immune system. IL-33 has long been studied in the context of Th2-related immunopathologies, such as allergic diseases and parasitic infections. However, its capacity to stimulate also Th1-type of immune responses is now well established. IL-33 binds to its specific receptor ST2 expressed by most immune cell populations, modulating a variety of responses. In cancer immunity, IL-33 can display both pro-tumoral and anti-tumoral functions, depending on the specific microenvironment. Recent findings indicate that IL-33 can effectively stimulate immune effector cells (NK and $CD8^+$ T cells), eosinophils, basophils and type 2 innate lymphoid cells (ILC2) promoting direct and indirect anti-tumoral activities. In this review, we summarize the most recent advances on anti-tumor immune mechanisms operated by IL-33, including the modulation of immune checkpoint molecules, with the aim to understand its potential as a therapeutic target in cancer.

Keywords: IL-33, tumor microenvironment, tumor immunity, eosinophils, ILC2, CD8 T cells, immune checkpoints, basophils

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INTRODUCTION

Interleukin-33 (IL-33) was initially described by JP Girard's group as a nuclear factor from high endothelial venules (NF-HEV) (1). It was later rediscovered, by a computational sequence search, as an IL-1 family member (2). Although initially defined as an immune component of Th2 response, its pleiotropic contribution to the immune response has now emerged. Hence, IL-33 has been involved in different immune processes, such as inflammatory diseases, allergies, infections and cancer (3). IL-33 is expressed as a nuclear factor by different types of cells, such as endothelial cells,

Abbreviations: AML, acute myeloid leukemia; BMDC, bone marrow-derived DC; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cells; HMGB1, high-mobility group box 1 protein; IL-33, Interleukin-33; ILC2, type 2 innate lymphoid cells; LA, lactic acid; LDHA, lactate dehydrogenase A; PDAC, pancreatic ductal adenocarcinomas; PD-1, programmed cell death-1; Tc9, IL-9 producing $CD8^+$ T cells; TILC2, tumor-infiltrating ILC2; TME, tumor microenvironment.

fibroblasts, epithelial cells and other stromal cells (4). In the tumor microenvironment (TME), these cells, together with tumor cells and some immune infiltrating cells, are an important source of IL-33 (5, 6). Like high-mobility group box 1 protein (HMGB1), IL-33 is released outside the cell after stress or damage and acts as an alarmin that activates the immune response (7). Two different isoforms of IL-33 have been described: the IL-33 full-length form (IL-33 FL) and the IL-33 mature form (8, 9). Several inflammatory proteases, mostly derived from neutrophils and mast cells, can process IL-33 FL into the mature form, endowed with superior (10- to 30-fold) bioactivity (4). Since both neutrophils (10) and mast cells (11) are recruited in the TME, these proteases may be abundantly present thus amplifying IL-33 activity. On the other hand, the pro-inflammatory action of IL-33 may be controlled by oxidation (12) or proteolytic cleavage by apoptotic caspases (13), leading to IL-33 inactivation. Therefore, the balance between different proteases as well as the nature of tumor cell death (necrotic *vs* apoptotic) may dictate the activity of IL-33 within the TME.

IL-33 binds to a heterodimer formed by its primary receptor ST2 and the co-receptor IL-1 receptor accessory protein (IL1RAP). This activates a signal cascade through MyD88-IRAK-dependent pathway, and leads to NF-κB, c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK) activation (2), which results in the release of a plethora of soluble mediators by different immune cells (14). IL1RAP is constitutively expressed at low levels by virtually all cells, including immune cells (15). ST2 is expressed primarily by cells involved in Th2 response, such as Th2 cells, eosinophils, basophils, mast cells, a subset of regulatory T cells (Treg) and type 2 innate lymphoid cells (ILC2), but also by Th1 cells, $CD8^+$ T cells, NK cells, macrophages, neutrophils, dendritic cells (DC) and B cells (16, 17). A soluble form of ST2 (sST2) exists as a decoy receptor that prevents IL-33 binding to the transmembrane receptor (18). Tumor, epithelial and immune cells express sST2 at various levels, which may contribute to regulate the availability of IL-33 in the TME (19).

The IL-33/ST2 axis has a controversial role in cancer immunity, since both pro- and anti-tumoral functions have been reported. This dichotomy seems to depend on multiple factors, such as the composition of the TME and tissue of tumor origin, and has been reviewed recently (16). In this mini review, we will focus on the anti-tumor effects of IL-33/ST2, with emphasis on the most recent advances on immune mechanisms and their potential exploitation for future therapeutic options.

IL-33 PROMOTES THE EFFECTOR FUNCTIONS OF $CD8^+$ T AND NK CELLS

Several studies demonstrated that IL-33 expression positively correlates with $CD8^+$ T and NK cell recruitment and activation in the TME. Transgenic expression of IL-33 in B16 or 4T1 tumor cells (20) or in the host (21), as well as exogenous administration of the recombinant protein (22) induce the recruitment of activated (IFN-γ^+ $CD107^+$) $CD8^+$ T and NK cells in the TME, which inhibited tumor growth in mice. In a breast cancer model, IL-33 induced the recruitment and activation of NK cells to the lung that prevented pulmonary metastasis onset (23). IL-33 can increase the cytotoxicity of $CD8^+$ T cells and NK cells also *in vitro*, indicating a direct action (21). Both FL and mature IL-33 isoforms acted as adjuvants in an HPV DNA vaccination model promoting antigen-specific $CD8^+$ T cell expansion and degranulation that resulted in regression of established TC-1 lung tumors (24). Although these findings point to a similar biological activity of FL and mature IL-33 isoforms, the possibility that FL IL-33 is converted into the mature form once released in the TME and exposed to local proteases cannot be excluded (9, 24).

Mechanistically, the ability of IL-33 to induce tumor-reactive IFN-γ^+ $CD107^+$ $CD8^+$ T and NK cells was recently shown to be dependent on MyD88 signaling in a mouse model of Lewis lung carcinoma (25). Furthermore, the IFN-inducing DNA sensor STING promoted tumor cytotoxicity by stimulating some chemokines (CXCL10 and CCL5) and IL-33, which participated in NK cell infiltration and activation in a mouse model of NK-sensitive melanoma (26). These studies reveal a possible link between IL-33 and IFN-related response in cancer immunity, as already reported in IgG4-related autoimmune diseases (27).

The role of endogenous IL-33 in mediating $CD8^+$ T cell-dependent antitumor responses was also demonstrated. In murine hepatocellular carcinoma, tumor-derived IL-33 promoted the expansion of IFN-γ^+ $CD4^+$ and $CD8^+$ T cells, increased CTL cytotoxicity and inhibited tumor growth (28). Induction of IL-33 production by stromal cells following LCMV-based vector immunotherapy elicited protective anti-tumor $CD8^+$ T cell effector responses (29). In a colon carcinoma model, endogenous IL-33 promoted IFN-γ expression by both $CD4^+$ and $CD8^+$ T cells, increased $CD8^+$ T cell infiltration over Treg cells and augmented $CD8^+$ T cell-mediated antitumor responses (30). These observations imply that endogenous levels of IL-33 by tumor and stromal cells may support cancer immune surveillance by $CD8^+$ T cells.

IL-33 can promote the effector functions of $CD8^+$ T cells also through stimulation of DC. IL-33 administration in tumor-bearing mice activated DC and increased Ag cross-presentation to $CD8^+$ T cells in melanoma (31) and acute myeloid leukemia (AML) models (32). One group reported that IL-33-stimulated DC expand a population of cytotoxic IL-9 producing $CD8^+$ T cells, termed Tc9, endowed with potent anti-tumor activity in melanoma-bearing mice (33). The relevance of Tc9 cells in human cancers is still unclear.

Notably, IL-33 is implicated in the differentiation of T cells into tissue-resident memory T (T_{RM}) cells, a recently identified $CD8^+$ T cell population found in various human cancers and correlating with favorable outcome (34). These cells express the integrins CD103 and CD49a and the C-type lectin CD69, and are characterized by *in situ* proliferation, location and persistence in close contact with malignant cells, *via* binding of CD103 to tumor E-cadherin (35). Whether and how IL-33 can affect T_{RM} in cancer warrants investigation.

MODULATION OF CD4$^+$ T CELL FUNCTIONS BY IL-33 IN THE TME

Both conventional and regulatory CD4$^+$ T cells are direct targets of IL-33. IL-33 can promote the recruitment and the immunosuppressive functions of Treg cells expressing ST2, favoring tumor growth and immunoevasion (36–39). On the other hand, IL-33 can activate conventional Th cells, inducing their phenotypic polarization, clonal expansion, and cytokine production (40). IL-33 preferentially promotes Th2 response, which is classically believed to contrast tumor immunity, although its role appears ambivalent (41). Under some conditions, such as in the presence of IL-12, IL-33 can induce Th1 responses (42, 43). In an HPV-associated mouse tumor model, IL-33 promoted IFN-γ and TNF-α production by antigen-specific CD4$^+$ T cells (24). Similarly, IL-33 was reported to amplify IFN-γ^+ CD4$^+$ T cells in mouse models of hepatocellular (28) and colon carcinoma (30, 44). These data demonstrated that IL-33 has the capacity to promote Th1-mediated anti-tumor response.

Lastly, IL-33 also promotes the differentiation of IL-9-producing Th cells (45), which exert potent antitumor activity in certain solid cancers, such as melanoma (46). Therefore, IL-33 can differently regulate CD4$^+$ T cell polarization and function in the TME. A comprehensive analysis of cytokine profiles activated by IL-33 in various cancers may help clarify the CD4$^+$ T cell subsets (including Treg) targeted by IL-33 in relation to the specific TME and anti-tumor response elicited.

IL-33 ACTIVATES EOSINOPHILS, BASOPHILS, AND MAST CELLS

Eosinophils infiltrate most human and experimental cancers where they play diverse roles (47). Migration to the TME can be mediated by eotaxins (eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26) that bind the CCR3 receptor highly expressed on eosinophils (47, 48) and by alarmins (i.e., HMGB1 and IL-33) released from dying tumor cells (22, 49). Whereas HMGB1 is a direct chemoattractant for eosinophils (50), IL-33 appears to recruit eosinophils only indirectly, *via* stimulation of tumor-released chemokines, such as CCL24 (51, 52), or through the activation of IL-5 producing ILC2 (53–55) and mast cells (56).

Several studies demonstrated the role of eosinophils in mediating the anti-tumoral activities of IL-33. Injection (22) or tumor expression (57) of IL-33 in melanoma-bearing mice inhibited tumor growth and this effect was abolished upon eosinophil depletion by injections of anti-Siglec-F mAb. In models of transplantable and colitis-associated colorectal cancer, tumor growth reduction induced by IL-33 was abrogated in eosinophil-deficient ΔdblGATA-1 mice, but was restored by adoptive transfer of eosinophils activated with IL-33 *ex vivo* (52). Mechanistically, eosinophils can exert anti-tumor activity partly by promoting the recruitment of CD8 T cells (22, 58). In fact, eosinophils are an important source of chemokines (CCL5, CXCL9, CXCL10) that attract CD8$^+$ T cells in TME (58) and can be up-regulated by administration of IL-33 (22). Moreover, eosinophils can exert direct tumor cytotoxicity (22, 51, 52). In a model of pulmonary melanoma metastasis, eosinophil depletion caused the inhibition of metastasis formation in mice receiving IL-33, without apparent involvement of cytotoxic CD8$^+$ T cells, thus suggesting an active role of eosinophils in the lung (22). In fact, IL-33 can directly activate human (59, 60) and mouse (52, 61) eosinophils by up-regulating activation markers (i.e. CD69), adhesion molecules (i.e., ICAM-1 and CD11b/CD18), and the degranulation markers CD63 and CD107a, resulting in the killing of several tumor cell types (51, 52, 62, 63). Once activated with IL-33, these granulocytes exert tumor cytotoxic functions through contact-dependent degranulation, involving polarization of eosinophilic effector proteins (eosinophil cationic protein, eosinophil peroxidase, and granzyme B) and convergence of lytic granules to the immunological synapses (51). This study provides the first evidence that eosinophils during degranulation employ a mechanism similar to that used by NK cells (64).

IL-33 is able to activate murine and human basophils, increasing histamine and cytokine production *in vitro* and promoting their expansion *in vivo* (16, 65–67). IL-33 can synergize with IL-3 to induce IL-9 production in human basophils (68), which may support tumor immunity (69). In human basophils, IL-33 alone does not directly induce degranulation but can enhance IL-3- and anti-IgE-mediated degranulation (67, 70). Recently, our group reported that mouse basophils stimulated with IL-33 up-regulate the expression of granzyme B and of the degranulation marker CD63 and induce melanoma cell killing *in vitro* (71). Although the role of basophils in cancer immunity is still unclear (72), this latter observation may broaden the spectrum of immune effector cells that can be activated by IL-33 within the TME.

Mast cells infiltrate several types of experimental and human tumors (56, 73). IL-33 activates human mast cells to release several cytokines (74) and enhances immune complex-triggered activation of human mast cells (75). Furthermore, IL-33 increases the expression of ICAM-1 (76) and MHC-II (77), and promotes the survival (78) and degranulation (79) of murine mast cells. However, due to the wide range of mediators they release, it is difficult to define the pro- or anti-tumorigenic activity of mast cells (11).

IL-33 AS AN ENHANCER OF ANTI-TUMOR ACTIVITIES OF ILC2

ILC2 constitutively express ST2 and respond directly to IL-33, which is necessary for their expansion, recruitment and activation (80, 81). Two distinct subsets of ILC2 have been described: resident natural ILC2 and inflammatory ILC2, which can be induced upon IL-33 stimulation (81). High numbers of ILC2 can be found in many IL-33-enriched tumors, although their role in cancer immunity remains controversial (82). Ikutani et al. first described an anti-tumoral

role of ILC2 in a mouse model of melanoma. In this study, systemic IL-33 injections expanded IL-5-producing ILC2 that induced eosinophil recruitment, which were critical to suppress pulmonary metastases (54). In another study, inoculation of IL-33-expressing EL4, CT26 or B16.F10 tumor cells induced MyD88-dependent intratumoral expansion of ILC2 in mice that were indispensable for IL-33-mediated antitumor activity independently of eosinophils (83). In this model, ILC2 exerted anti-tumoral activity through production of CXCL1 and CXCL2. Binding of these chemokines to tumor cell-expressed CXCR2, which was sustained by the hypoxic TME created by IL-33, resulted in tumor cell apoptosis. This study first demonstrated that activated ILC2 can be cytotoxic for tumor cells.

A recent study on the B16.F10 melanoma model showed that TME acidification caused by lactic acid (LA) produced by the tumor impaired ILC2 survival and function (55). This prevented tumor infiltration of ILC2 and resulted in rapid tumor growth. Accordingly, gene expression analysis in human cutaneous melanomas revealed an inverse correlation between lactate dehydrogenase A (LDHA, the enzyme responsible for LA production) and markers associated with ILC2. *In vivo* interference with LDHA in B16.F10 tumors or administration of IL-33 to tumor-bearing mice increased the number of intratumoral ILC2 and restored ILC2 ability to contrast tumor progression. IL-33 also induced an increase in the number of tumor infiltrating eosinophils. This study reveals an anti-tumorigenic role of IL-33/ILC2/eosinophils axis controlled by glucose metabolism.

Moral and co-workers reported that ILC2 infiltrate human and mouse pancreatic ductal adenocarcinomas (PDAC) (84). High frequencies of tumor-infiltrating ILC2 (TILC2) were found in "hot" tumors (enriched in $CD8^+$ T cells), and correlated with better survival and high expression of IL-33. By comparing the effects of IL-33 deficiency (or exogenous administration) on orthotopic PDAC and heterotopic skin tumor growth, the authors demonstrated that TILC2 have tissue-specific effects on PDAC immunity that depended on IL-33/ST2. In fact, pancreatic TILC2, unlike skin TILC2, expressed ST2 and responded to IL-33. In orthotopic PDAC, IL-33/TILC2 axis primed tissue-specific $CD8^+$ T cell immunity through recruitment of cross-presenting $CD103^+$ DC.

Overall, these studies suggest that despite the divergent effects of ILC2 in tumor immunity, proper activation, such as with IL-33/ST2 stimulation, may promote the anti-tumor functions of these cells through multiple mechanisms, including recruitment of eosinophils and cross-presenting DCs, and tumor cytotoxicity. Given the tissue-specific phenotypes of ILC2, it is possible that such mechanisms may vary depending on the tissue of tumor origin.

MODULATION OF IMMUNE CHECKPOINTS BY IL-33

Cancer immunotherapy targeting immune checkpoints has proven effective in treating "hot" tumors through the restoration of preexisting T cell responses. Programmed cell death-1 (PD-1) promotes apoptosis of antigen-specific T-cells, while it sustains regulatory T cell development and function (85, 86). In the TME, up-regulation of PD-1 on T cells occurs in response to activation due to tumor antigens (87), while overexpression of its ligands (PD-L1 and PD-L2) on cancer cells is a well-known immune escape mechanism (88). PD-1 is expressed on a variety of different immune cell types, such as T cells, B cells, NK, myeloid cells, mast cells and innate lymphoid cells (89, 90). Mouse ILC2 express PD-1 in different percentages depending on their tissue of origin and its expression is enhanced by IL-33 stimulation, resulting in impaired Th2-type cytokine production (91, 92). In a mouse model of obesity, TNF-α triggered the expression of IL-33 by pre-adipocytes, which was responsible for PD-1 upregulation on ILC2 (92). Interaction between $PD\text{-}1^+$ ILC2 and $PD\text{-}L1^{hi}$ M1 macrophages resulted in impaired production of IL-5 and IL-13 by ILC2. These findings point to a role of IL-33 in PD-1/PD-L1 pathway.

Emerging data indicate that IL-33 may modulate the PD-1/PD-L1 axis also in cancer. In an AML model, Qin et al. observed that IL-33 induced not only an increase of PD-1 expression on $CD8^+$ T cells in peripheral blood, but also higher levels of PD-L1 on tumor cells (32). IL-33 treatment combined with PD-1 blockade prolonged the survival of leukemic mice, providing the first evidence that IL-33 may increase the therapeutic efficacy of immune checkpoint inhibitors. Recently, Moral et al. carried out similar studies on the PDAC mouse model. They showed that IL-33 treatment increased the expression of PD-1 on TILC2, but not in draining LN ILC2, indicating selective activation in the tumor immune compartment (84). Combination of IL-33 and anti-PD-1 reduced tumor growth and improved the survival of PDAC mice in an ILC2-dependent fashion. Of note, this study demonstrated that IL-33 activated TILC2 were direct targets of anti-PD-1. Thus, activation of ILC2s with IL-33 may be a strategy to increase immunotherapy efficacy in ILC2-infiltrated cancers.

IL-33 can affect PD-1/PD-L1 signaling in other immune cells. In a breast cancer model, IL-33 administration increased the percentage of $NKp46^{1+}$ $PD\text{-}1^+$ cells in the TME, while these cells were less frequent in ST2-deficient mice (93). Furthermore, in the B16.OVA melanoma model, systemic administration of IL-33 combined with injection of dectin-1-activated bone marrow-derived DC induced activation and PD-1 expression in OVA-specific $CD4^+$ T cells (45). The same group reported that administration of IL-33 reduced the expression of the checkpoint molecules PD-1, LAG-3 and 2B4 on $CD8^+$ T cells in mice immunized with "resting" DC (33). Although these two studies suggest that the modulation of immune checkpoints in T cells by IL-33 occurs *via* stimulation of DC, the possibility that IL-33 could also directly activate T cells cannot be excluded. Overall, these findings suggest that IL-33 can affect the PD-1 pathway in several immune cells. Understanding the mechanisms by which IL-33 targets PD-1 in various cancer types may help improving immunotherapy protocols.

The role of IL-33 in the modulation of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathway has been less explored.

CTLA-4 is constitutively expressed in regulatory T cells and it is up-regulated in conventional T cells upon activation, where it functions as an inhibitory signal of T cell response (94). In a B16.F1 melanoma pulmonary metastasis model, IL-33 increased the frequency of $CD8^+$ T cells expressing PD-1, KLRG-1 and CTLA-4 (95). Hollande et al. reported that tumors expressing high levels of endogenous IL-33 (i.e., Hepa 1-6 and EMT6) respond to combined CTLA-4/PD-1 blockade partially through the help of eosinophils (57). Although this study does not directly address whether IL-33 is relevant for up-regulation of these immune checkpoint molecules, it suggests that local IL-33 and eosinophils recruitment in the TME may promote immunotherapy efficacy. This hypothesis is supported by an increasing number of reports that show a positive correlation between eosinophilia and clinical response to anti-PD-1 and anti-CTLA-4 in cancer patients (47, 96, 97).

CONCLUDING REMARKS

Although the role of IL-33 in cancer immunity remains controversial, it appears that this alarmin has beneficial effects in certain types of experimental tumors, particularly melanoma (16, 20–22, 31, 51, 57). The current literature suggests that the anti-tumor properties of IL-33 are attributable to its capacity to stimulate $CD8^+$ T cells, NK, DC, eosinophils and ILC2 (**Figure 1**). Eosinophils are recruited early in the TME and may play a role in the first containment of tumor development (98). A similar function may be potentially played by ILC2, mast cells and basophils. Although relatively rare in human cancers, these cells can release several soluble mediators that may orchestrate tumor immunity in various manners (11, 47, 56, 71, 72, 82). For example, following stimulation with IL-33, eosinophils and

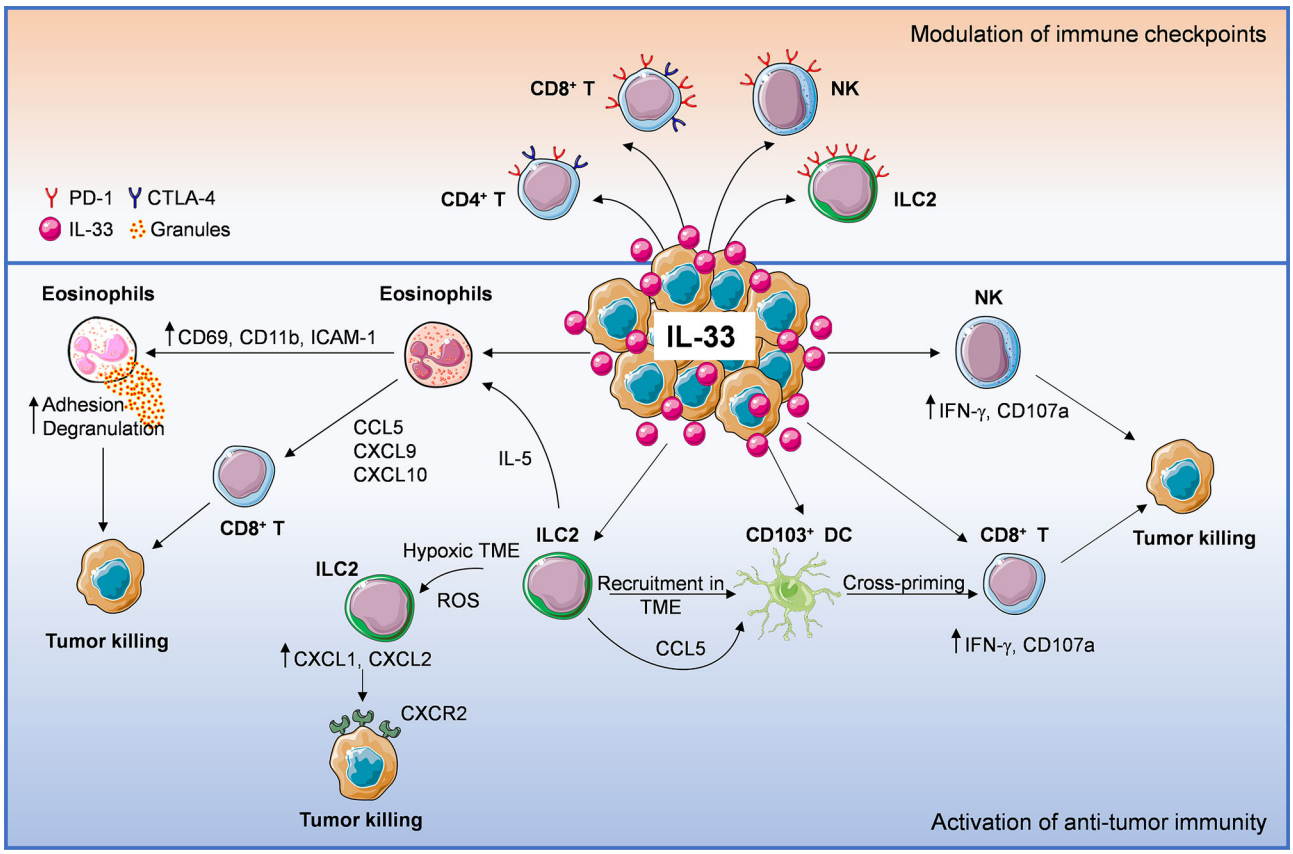

FIGURE 1 | Anti-tumoral mechanisms of interleukin-33 (IL-33) in the tumor microenvironment (TME). IL-33 administration or its physiological expression within the TME leads to direct or indirect recruitment of several immune effector cells such as eosinophils, ILC2, DC, NK cells, $CD8^+$, and $CD4^+$ T cells, establishing an immune cross-talk or directly controlling tumor growth. ILC2 cells can: 1) directly induce tumor cell killing through CXCL1/CXCL2 release and binding to tumoral CXCR2, 2) promote the recruitment of eosinophils *via* IL-5 production, 3) release CCL5 that facilitates $CD103^+$ DC recruitment and cross-priming of $CD8^+$ T cells. Following IL-33 exposure, eosinophil recruitment may result in: 1) direct tumor cell killing *via* adhesion-dependent degranulation and 2) release of $CD8^+$ T cell-attracting chemokines (CCL5, CXCL9, CXCL10). Moreover, IL-33 can activate NK, $CD8^+$ T (directly or *via* stimulation of cross-presenting DC) and $CD4^+$ T cells, promoting anti-tumor effector responses. These events may be hindered by concomitant recruitment of $ST2^+$ Treg cells. Lastly, IL-33 also up-regulates programmed cell death-1 (PD-1) on T lymphocytes (especially $CD8^+$ T), NK cells and ILC2, as well as CTLA-4 on T cells, suggesting that this cytokine may improve the therapeutic response to immune checkpoint inhibitors.

ILC2 produce chemokines attracting $CD8^+$ T cells (22) and DCs (84), respectively, thus contributing to the initiation of adaptive responses. Furthermore, release of Th2 cytokines, (i.e., IL-4 and IL-5) by basophils, mast cells and ILC2 may promote the recruitment of eosinophils and macrophages that control tumor progression (99, 100). Direct stimulation of NK, $CD8^+$ and $CD4^+$ T cells by IL-33 has been reported to promote Th1-associated anti-tumor responses in several tumor models (20, 21, 23–26, 28–30). Induction of IL-9 producing $CD4^+$ (45) and $CD8^+$ (33) T cells by IL-33 may also contribute to anti-tumor immunity. However, IL-33 can induce and amplify Th2 responses in the TME, which may support tumor progression. Moreover, stimulation of $ST2^+$ Treg cell recruitment in the TME (3, 16) may further dampen anti-tumor responses. Therefore, tissue-specific environmental factors that shape the local immune TME may dictate the balance of immune responses induced by IL-33. This aspect should be carefully considered when harnessing the IL-33/ST2 axis in tumors particularly enriched in Treg cells, such as breast, lung and gastrointestinal cancers (101).

IL-33 appears to increase the expression of PD-1/PD-L1 and CTLA-4 molecules on certain immune cells (**Figure 1**) and to improve immunotherapy efficacy of checkpoint blockade in some cancer models. The modulation of these and other checkpoint molecules by IL-33 and the immune targets in each cancer type remain to be fully elucidated. In this view, targeting IL-33/ST2 in specific immune cell populations may be a promising strategy to increase the therapeutic response to immune checkpoint inhibitors. Since T_{RM} cells express high levels of immune checkpoint molecules (i.e., PD-1, CTLA-4 and Tim-3), these cells are regarded as key targets of immune checkpoint inhibitors dictating immunotherapy efficacy (102). Future investigation should be directed to evaluate whether targeting the IL-33/ST2 pathway may increase the density of T_{RM} cells in the TME and improve the response to immune checkpoint blockade.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

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