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A role for an HTLV-1 vaccine?

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HTLV-1 is a global infection with 5-20 million infected individuals. Although only a minority of infected individuals develop myelopathy, lymphoproliferative malignancy, or inflammatory disorders, infection is associated with immunosuppression and shorter survival. Transmission of HTLV-1 is through contaminated blood or needles, mother-to-child exposure through breast-feeding, and sexual intercourse. HTLV-1 is a delta retrovirus that expresses immunogenic Gag, Envelope, TAX, and Hbz proteins. Neutralizing antibodies have been identified directed against the surface envelope protein, and cytotoxic T-cell epitopes within TAX have been characterized. Thus far, there have been few investigations of vaccines directed against each of these proteins, with limited responses, thus far. However, with new technologies developed in the last few years, a renewed investigation is warranted in search for a safe and effective HTLV-1 vaccine.

KEYWORDS

HTLV 1, TAX, envelope (E), glycoprotein (GP), vaccine, neutralizing abs, cytotoxic T cell

HTLV-1

HTLV-1 is prevalent in many parts of the world, including Central & South America, Caribbean Islands, Africa, northeast Iran, southern Japan, Melanesia, Australia, where endemic rates are 5-10%, but in some isolated communities endemic rates as high as 50% have been identified (1, 2). However, several large and highly populated regions in India and North and East Africa have not been screened. In the US, the prevalence of HTLV infection is 0.1-0.2% (3). HTLV-1 strains are highly conserved with <2% overall divergence for cosmopolitan strains (HTLV-1a) from most areas of the world, with up to 10% divergence with strains from Australia and Melanesia (HTLV-1c)(Figure 1) (4). Strains from central Africa (HTLV-1b, d-g) are somewhat more divergent than the HTLV-1a strains.

Diseases caused by HTLV-1 include an aggressive CD4+ lymphoproliferative malignancy, designated adult T-cell leukemia lymphoma (ATLL) and spastic paraparesis, known as HTLV-1 myelopathy (HAM) or tropical spastic paraparesis (TSP) (5). Although, only 5-10% of infected individuals develop these disorders, HTLV-1 is associated with many other clinical inflammatory disorders, immunosuppression, and shortened survival (6). Sequence differences in the virus do not correlate with disease development (7). HTLV-1 causes a lifelong persistent infection,

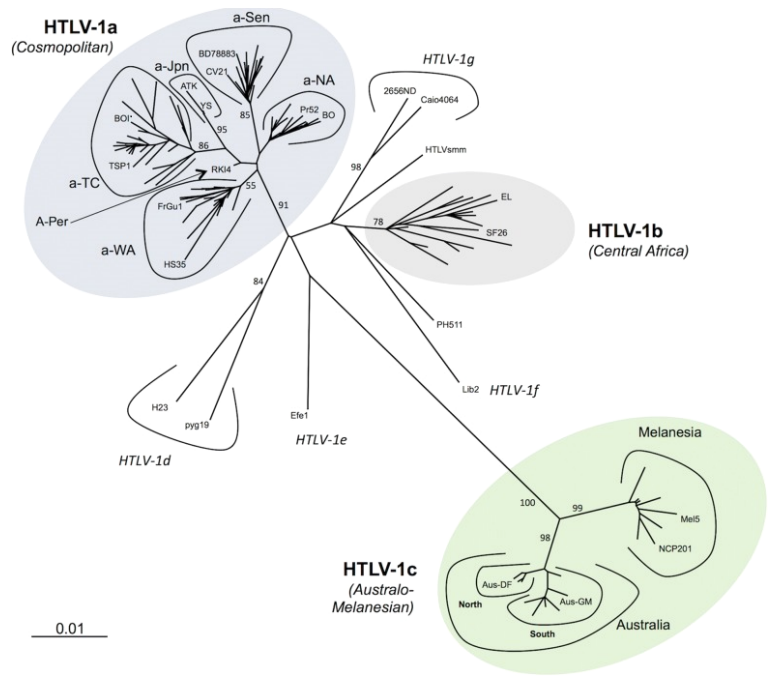

FIGURE 1
Phylogenetic representation of the HTLV-1 genotypes and subgroups. An alignment of complete LTR sequences (774 nucleotides long) from 178 HTLV-1 strains was obtained. The unrooted phylogenetic tree was generated with the neighbor-joining method using the GTR model (gamma=0.50). Branch lengths are drawn to scale, with the bar indicating 0.01 nucleotide replacements per site. Numbers on each node indicate the percentage of bootstrap samples (of 1000 replicates). HTLV-1 genotypes (a-g) and subgroups with HTLV-1a and HTLV-1c are presented. Published with permission from the author and journal (4).

which is never truly silent (8). This led to an increased focus by the WHO on HTLV epidemiology and prevention strategies, which included a recommendation to develop a global strategy for the elimination of HTLV-1 (9).

Transmission of HTLV-1 is through contaminated blood or needles, sexual intercourse, and breast feeding (10). Rarely, HTLV is transmitted through ritual scarification practices (11). Zoonotic transmission through severe bites from simian T-cell leukemia virus type 1 infected non-human primates have also occurred among hunters in central Africa (12). Transmission of HTLV-1 from mother-to-child can be reduced by screening and education, which is a nationwide strategy in Japan (13). Horizontal transmission of HTLV-1, by sexual intercourse or blood transfusion is also preventable (14). An additional emerging concern is HTLV-1 infection upon organ transplantation (15). Attempts to facilitate screening remain to be developed.

After transmission, a balance between virus replication, expansion of infected cells, and immune response to the virus leads to the establishment of a proviral load "set point" after HTLV-1 acquisition. However, there have been few studies of acute HTLV-1 infection aimed at assessing the determinants and kinetics of the set point. In a study of three individuals who acquired HTLV-1 infection after organ transplantation, the proviral load set point was reached within 6 weeks (15). Thus, therapeutic approaches to infection prophylaxis have a limited time window in which to act. Nevertheless, it remains unclear whether transmission through other routes establishes the proviral set point with similar kinetics.

Cellular transfer of virus occurs more commonly *via* cell-to-cell contacts than *via* free virus particles (16). Two types of cell-cell contacts have been described to be critical for HTLV-1 transmission, tight junctions and cellular conduits (17). Non-exclusive mechanisms of virus transmission at cell-cell contacts include polarized budding into synaptic clefts and cell surface transfer of viral biofilms at virological synapses (18, 19). In contrast to CD4+ T-cells, dendritic cells can be infected with cell-free virus and, to a greater extent, *via* viral biofilms (20).

HTLV-1 is a member of the δ retrovirus family, which also includes HTLV-2 and bovine leukemia virus (BLV) (21). HTLV-2 is not clearly associated with disease, whereas BLV is a cause of B cell lymphoproliferative disorders in cattle. HTLV-1 encodes classical retrovirus structural proteins from group-specific antigen (*gag*) and envelope (*env*) genes, and enzymes from the protease (*pr*) and polymerase (*pol*) genes that encode the viral protease, reverse transcriptase (RT), and integrase (IN). Virus infection is mediated by a receptor complex on the cell surface and the virus particle is taken into cells by membrane fusion.

Virus uncoating activates RT to copy the two copies of the plus-strand viral RNA genome into a dsDNA copy that is integrated into cellular DNA by the viral IN. Transcription is mediated by cellular RNA polymerase II, and viral RNAs may be spliced or unspliced prior to export from the nucleus. Translation produces viral proteins, including the Gag, Gag-Pr, and Gag-Pr-Pol polyprotein precursors which are processed into individual components during virus budding. Envelope is synthesized and processed in the reticuloendothelial-Golgi system, transported to the plasma membrane, and incorporated into the budding virus. Regulatory proteins, are encoded from multiple spliced RNAs and include the transactivator protein, TAX, the regulator of splicing and nuclear export, REX, the helix zipper protein, HBZ, as well as proteins designated p12, p27, and p30 that are presumed to regulate virus replication and/or pathogenesis.

HTLV-1 encodes two oncoproteins, TAX and HBZ (22–24). TAX promotes cytoplasmic signaling through various receptors and causes abnormal cell cycle regulation, genetic instability, and inhibition of DNA repair and apoptosis (25). HBZ counteracts the functions of TAX promoting a persistent latent infection (26). HBZ regulates signaling pathways important for inflammation, transcription, apoptosis, autophagy, histone methylation, and T-cell differentiation (27).

HTLV-1 envelope glycoprotein

The HTLV-1 *env* gene encodes the gp62, 488 amino acid envelope precursor glycoprotein which is cleaved by furin-like enzymes into a 20 amino acid (AA) signal peptide, a 292 amino acid gp45 surface envelope protein (SU) and a 176 amino acid gp21 transmembrane envelope protein (TM). SU has four asparagine (N)-linked glycosylation sites (AA 140, 222, 244, and 272; Figure 2), and TM has a single N-linked glycosylation site (AA 404), a disulfide bond (AA 393-400), and a S-palmitylated cysteine residue (AA 462). TM includes the fusion peptide (AA 313-333), and two coiled coil, heptad repeat (HR) domains (AA 341-387 and 397-429).

SU mediates infection by binding to cellular entry factors heparin sulfate proteoglycans (HSPG), glucose transporter 1 (GLUT-1), and neuropilin-1 (NRP-1) (28). Subsequent conformational changes include isomerization of a SU-TM intersubunit disulfide result in fusion of the viral and cellular membranes (29). Although the crystal structure for SU has not yet been determined, it has been proposed to contain two separate folding domains separated by a proline-rich linker peptide (PRR; Figure 2) (30). The N-terminal region, designated the receptor-binding domain (RBD) is necessary and sufficient for binding to GLUT-1. The C-terminal domain includes the binding site for HSPG.

Retroviral envelope proteins, like those of coronaviruses, arenaviruses, filoviruses, pneumoviruses, and orthomyxoviruses are class I fusion proteins (31). They are all type I single-pass trans-membrane proteins that form non-covalently linked homotrimers in their pre- and post-fusion conformations. The structural hallmark of class I fusion proteins is the parallel trimeric α-helical coiled-coil in the post-fusion C-terminal subunit (TM in the case of retroviruses). This long α-helix of the coiled coil contains the HR with non-polar amino acids at positions 1 and 4 of the repeats. The C-terminal portion of the α-helix runs antiparallel to the N-terminal portion along the grooves of the coiled-coil to complete the post-fusion hairpin, thereby making a trimeric postfusion six-helix bundle. Detailed structures are available for many of these proteins in each conformation. A common feature of these proteins is their dynamic conformational changes, presenting open and closed forms in equilibrium (32). There is a large body of research indicating that it is the closed form that is recognized by the known broadly neutralizing antibodies, whereas the epitopes exposed in the open form which do not bind these antibodies

Neutralizing anti-HTLV-1 antibodies

Polyclonal and monoclonal antibodies to HTLV-I envelope proteins, including SU, have been demonstrated to neutralize HTLV-I infection (33–36). Neutralizing antibodies were observed in HTLV-1 infected individuals, which can prevent infection (37–40). These antibodies may interfere with receptor or coreceptor recruitment, or prevent receptor-induced changes in SU conformation that are required to activate the fusogenic properties of envelope (41). Major neutralizing domains of SU have been mapped to 4 domains (AA 53-75, 88-107, 175-215, and 287-311, Figure 2) (35, 36, 42). Efficacy of neutralizing antibodies was demonstrated by passive transfer of anti-envelope antibodies which block blood-borne or milk-borne HTLV-1 infection of rabbits (43–47)

HTLV-1-specific cellular immunity

HTLV-1 infection elicits a strong CTL response (48). The frequency of HTLV-1-specific CTLs may be very high with up to 10% of circulating CD8+ T cells recognizing a single immunodominant CTL antigen target, TAX (49). The frequency of HTLV-1-specific CTLs is correlated with the HTLV-1 proviral load (50). This raised the hypothesis that HTLV-1-specific CTLs may fail to eradicate the virus, and may contribute to the inflammatory tissue damage with disease. presentation However, there is also evidence that CTL responses to HTLV-1 may be protective. Higher expression of CTL effector proteins is correlated with lower proviral load (51). In addition, the immunodominant TAX protein is subject to positive selection *in vivo* (52). Envelope-specific CTLs have also been detected, but are present at low frequencies (53, 54). T-cell

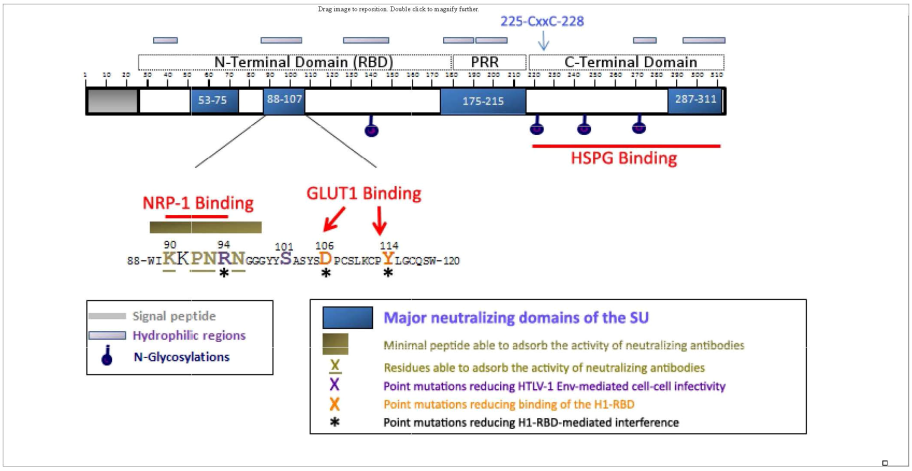

FIGURE 2
Localization of the neutralizing regions and the domains and residues involved in HSPGs, NRP-1, and GLUT-1 binding within the HTLV-1 SU protein. The 90-94 motif identified as critical for direct NRP-1 binding corresponds to a minimal neutralizing epitope, and contains the R94 residue required for HTLV-1 particle infectivity. R94, as well as D106 and Y114 that mediate binding of the H1-RBD to target cells are required for H1-RBD-mediated receptor interference. The C-terminal domain of the SU contains the determinants for HSPG binding. Published with permission from the author and the journal (28).

epitopes within envelope in HLA-A2-positive individuals were mapped to residues 175-183, 182-190, 239-247, 395-403, and 442-450 (55, 56). The lytic efficiency of the CD8+ T cell response, as measured by the fraction of HTLV-1-expressing cells eliminated per CD8+ cell per day, was found to be inversely correlated with both the proviral load and the rate of spontaneous proviral expression (57). However, ATLL patients have weak CTL responses to HTLV-1 antigens (58).

Vaccination against HTLV-1

Preventative vaccines are highly effective against a wide range of viral diseases, including cancer viruses, hepatitis B virus and human papilloma virus (59). There has also been a resurgence in therapeutic cancer vaccines (60), including malignancies caused by viruses (61), as well as non-viral cancers associated with production of neoantigens (62). However, most efforts focused on a vaccines for retroviruses have been concerned with HIV, and with very limited success, thus far (63). Nevertheless, an inactivated viral vaccine for feline leukemia virus has been developed that provides protection against heterologous strain infection (64). The vaccine did not prevent infection, but it did induce an antibody response and results in proviral loads more than 100-fold lower than challenged but non-vaccinated cats, that were detectable for a shorter time interval. A recombinant feline leukemia virus vaccine (PureVAX) is also commercially available, which utilizes a canary pox vector to express a mutated envelope, gag, and a truncated polymerase protein, resulting in 93% efficacy (65)

Several studies have evaluated vaccines against BLV (66). Inactivated BLV, crude lysates from persistently infected cell lines, peptide antigens, poxvirus-expressed BLV antigens, or DNA vaccines led to partial protection, with declining neutralizing antibody titers and poor stimulation of CTL responses. An attenuated BLV vaccine was made with deletions in TAX and the antisense accessory (G4) genes, resulting in a virus capable of very low levels of infectivity and replication, which is commercially available (67).

Kazanji et al. tested a chimeric peptide vaccine composed of B- and T-cell epitopes of HTLV-1 (68). They identified high titer antibodies and a high frequency of interferon γ-expressing cells against the envelope and TAX immunogens, but not against individual TAX peptides. After challenge, partial protection was achieved as evidenced by lower proviral loads in immunized compared to control animals. Studies with peptide immunization had previously been performed in rabbits by Takehara et al. (43). In that case, rabbits were vaccinated with a peptide corresponding to Env amino acids 175-196. Although pre-challenge sera from these rabbits showed high titers of anti-HTLV-1 Env antibodies, after challenge, virus was recovered from all rabbits.

A highly attenuated poxvirus expressing the entire HTLV-1 envelope protein was used to immunize New Zealand white (NZW) rabbits (69). The animals were protected from HTLV-1 infection, but not protected upon re-challenge 5 months later with 10- to 100-fold greater infectious virus load. It was unclear

whether the lack of protection to re-challenge was a result of being overwhelmed with a large dose of virus, or due to the presence of a viral variant that evaded the immune response. Hokada et al. also inoculated rabbits with a recombinant vaccinia virus carrying the HTLV-1 envelope gene (70). This vaccine elicited anti-envelope and antibodies, but neutralizing antibodies were not detected. In addition, this vaccine did not prevent infection.

Nakamura et al. immunized 4 cynomolgus monkeys with 4-6 doses of *E coli*-produced HTLV-1 envelope protein (71). These animals were protected against infection, when challenged with a live HTLV-1 producing cell line, MT2. However, 2 monkeys inoculated with fewer doses did not produce antibodies that block HTLV-1 induced syncytium formation and were not protected against challenge with MT2 cells.

Ibuki et al. inoculated 2 cynomolgus monkeys with a recombinant vaccinia virus expressing the HTLV-1 envelope, and elicited neutralizing antibodies, and these animals were protected against infection (72). Kazanji et al. tested several HTLV-1 vaccines in squirrel monkeys (73). These included attenutated vaccinia virus-derived HTLV-1 *env* and/or *gag* expression vectors. However, after 3 inoculations, only one of three animals was protected against infection. Since naked DNA has also been used to induce neutralizing antibodies against HTLV-1 envelope glycoproteins in mice (74, 75), Kazanji et al. also incorporated this reagent in their studies (73). Priming animals with an *env* DNA vaccine followed by the recombinant vaccinia virus expressing *env* and *gag* resulted in protection of all three inoculated animals.

Kabirit et al. synthesized a multi-epitope chimeric protein with TAX, Env, and Gag immunodominant epitopes encapsulated in biodegradation poly(D,L-lactide-co-glycolide) nanoparticles (76). This preparation elicited antibody and cytokine responses in mice, but efficacy against infection was not reported. Humoral and cell-mediated immune response against the HTLV-1 envelope were detected in the protected animals.

A recombinant HTLV-1 glycoprotein protein vaccine was made against the HTLV-1c glycoprotein (77). This subunit vaccine utilized a molecular trimerization domain clamp to stabilize the prefusion conformation of the glycoprotein (78). This approach was previously used to stabilize influenza A hemagglutinins, using HIV-1-derived heptad regions. For the HTLV-1c envelope vaccine, the clamp was modified to negate production of anti-HIV-1 envelope antibodies. Use of several different adjuvants resulted in strong antigen-specific responses in mice.

MHC-I-bound HTLV-1 peptides have been identified which give rise to HTLV-1-specific CTLs *in vivo* (79). A therapeutic vaccine to activate TAX-specific CTLs was developed using TAX peptide-pulsed dendritic cells, resulting in favorable clinical outcomes in three ATLL patients (80, 81). These investigators also demonstrated that dendritic cells from peripheral blood mononuclear cells of a chronic ATLL patient evoked TAX-specific CTL-responses (82). However, since half of all ATLL patients lose the ability to express TAX, this approach may have limited utility (83). MacNamara et al. quantified the contribution of all HLA class I alleles to host protection against infection with HTLV-1 (84). They concluded that CD8+ cell response to HBZ are most effective. HTLV-1 carriers who had MHC class 1 alleles which could preferentially bind and present epitopes from HBZ were more likely to have low proviral loads, and less likely to develop disease than carriers who had MHC alleles which weakly bound HBZ peptides. Vaccination with a recombinant vaccinia virus expressing HBZ resulted in CTLs with anti-lymphoma effects in HBZ transgenic mice (85). In addition, this vaccine produced HTLV-1-specific T cell responses in infected rhesus monkeys (85).

RNA vaccines

Messenger RNA (mRNA)-based vaccines hold the promise to revolutionize the infectious disease prevention field by addressing current manufacturing challenges and offering novel vaccine compositions (86). Critical quality attributes are high efficiency of expression with a 5'cap, 5'untranslated region of optimal length with key regulatory elements, codon optimization, 3'poly-A tail length appropriate for translation, and lack of impurities that induce inflammatory cytokines and reduce expression. The use of lipid nanoparticle (LNP) formulations stabilize the mRNA and facilitate cellular uptake. As of 2020, 12 clinical trials for mRNA-based infectious disease vaccines were completed for infectious agents including respiratory syncytia virus, rabies, chikungunya, zika, parainfluenza, influenza, and cytomegaloviruses. The recent success of coronavirus-19 mRNA vaccines has re-energized the field (87). These vaccines, based on a "universal" LNP delivery system, have proven tolerable and highly efficacious. Challenges remaining include thermal instability of the mRNA cargo, further optimization of the nanoscale delivery platform to produce target-specific immunoactivation and prolong the duration of the effect, achieving a "one-shot" approach, achieving low cost for low- to middle-income countries, lack of clarity about the longevity and type of immunoprotection offered, and hypersensitivity reactions. This approach is worthy of investigation for HTLV-1.

Clinical vaccine trials

A safe and effective vaccine preparation in animal studies will eventually be considered for clinical trials. This would include phase 1 safety and pharmacokinetics studies in volunteers. Phase 2 and 3 trials would then be targeted to a population at risk of acquisition of HTLV-1. The largest and most suitable population would likely be individuals at significant risk of sexual acquisition of HTLV-1 in an endemic region.

Conclusions

Previous studies in rabbits and monkeys suggest that inoculation with HTLV-1 gene products may provide protection against infection. The ideal vaccine candidate and method of inoculation remains to be deciphered. In addition, immune correlates of response remain to be determined. Although some studies suggest that neutralizing antibodies against the HTLV-1 envelope protein may provide protection against infection, the role of cytotoxic T lymphocyte responses against envelope and other viral proteins remains to be fully characterized. In addition, antibody-dependent cellular cytotoxicity, known to occur in primary infection (88–90), could also be important in vaccine protection.

The WHO issued a technical report in 2020 with a strong recommendation for global strategies to eliminate transmission of HTLV-1 (9). Thus, further studies of possible efficacy and safety of HTLV-1 vaccines is warranted. It will be interesting to determine why some people who are infected with HTLV-1, manage to maintain a very low proviral load set-point, and have a very low risk of disease. Analysis of such HTLV-1 "elite controllers" could provide important details to defining a protective response to the virus. If a safe and effective vaccine can be developed, it remains unclear which individuals might benefit from its use. Individuals at greatest risk of acquisition of HTLV-1, include people who are sexual partners of HTLV-1 infected subjects. In addition, a vaccine could have benefits in preventing maternal-to-child transfer. Although control of breast-feeding was effective in Japan in preventing vertical transmission, limiting breast-feeding in developing countries might cause malnutrition issues with newborns, so vaccination might have unique advantages in these settings. Thus, the highest prevalence of such individuals will be sexually active individuals in HTLV-1 endemic areas.

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

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Conflict of interest

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