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In vivo immunogenicity of Tax(11–19) epitope in HLA-A2/DTR transgenic mice: Implication for dendritic cell-based anti-HTLV-1 vaccine

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ABSTRACT

Viral oncoprotein Tax plays key roles in transformation of human T-cell leukemia virus (HTLV-1)-infected T cells leading to adult T-cell leukemia (ATL), and is the key antigen recognized during HTLV-associated myelopathy (HAM). In HLA-A2+ asymptomatic carriers as well as ATL and HAM patients, Tax(11–19) epitope exhibits immunodominance. Here, we evaluate CD8 T-cell immune response against this epitope in the presence and absence of dendritic cells (DCs) given the recent encouraging observations made with Phase 1 DC-based vaccine trial for ATL. To facilitate these studies, we first generated an HLA-A2/DTR hybrid mouse strain carrying the HLA-A2.1 and CD11c-DTR genes. We then studied CD8 T-cell immune response against Tax(11–19) epitope delivered in the absence or presence of Freund's adjuvant and/or DCs. Overall results demonstrate that naturally presented Tax epitope could initiate an antigen-specific CD8T cell response in vivo but failed to do so upon DC depletion. Presence of adjuvant potentiated Tax(11–19)-specific response. Elevated serum IL-6 levels coincided with depletion of DCs whereas decreased TGF-β was associated with adjuvant use. Thus, Tax(11–19) epitope is a potential candidate for the DC-based anti-HTLV-1 vaccine and the newly hybrid mouse strain could be used for investigating DC involvement in human class-I-restricted immune responses.

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1. Introduction

Human T-cell leukemia virus (HTLV-1) causes a spectrum of abnormalities, the most prominent being adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy (HAM) [1]. Leukemic cells are usually monoclonal with respect to the integration of HTLV-1 provirus, which is the end result of progression from polyclonality during the course of T-cell transformation with the establishment of malignant cell lineages [2,3]. A number of epitopes of Tax, the major HTLV-1 oncogenic transactivator protein, have been recognized by HTLV-1-specific $CD8^+$ CTLs in infected individuals carrying HLA-A2, -A11, or -A24 [4–10]. In $HLA\text{-}A2^+$ patients, the frequency of Tax-specific CTLs can be as high as 30% of all $CD8^+$ T-cells in the peripheral blood [11] and even higher in CSF [4,12,13], with Tax(11–19) epitope being the immunodominant epitope in asymptomatic carriers [14], ATL [7,15], and HAM

Abbreviations: AC, asymptomatic carrier; ATL, adult T-cell leukemia; CFSE, 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester; DC, dendritic cell; DT, diphtheria toxin; DTR, diphtheria toxin receptor; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; HTLV-1, human T-cell leukemia virus type 1; IFA, incomplete Freund's adjuvant; THP, tetanus helper peptide.

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[4,6,12,16–19]. During the development of ATL, general immune suppression has been reportedly due to dysfunction of Tax-specific CTLs [7,20]. In addition, both IFN-γ production and proliferative capacity of tetramer-binding Tax-specific CD8+ T-cells are severely impaired in ATL patients [21], and a Tax-low or Tax-negative phenotype is associated with clonal proliferation of ATL cells [22,23]. Consequently, it has been established that impairment of the Tax-specific response is critically involved in ATL induction and disease progression.

ATL is highly aggressive and malignant with the median survival time measured in months even with standard treatment such as chemotherapy, antiviral drugs, or interferon therapy [24]. Autologous Tax-specific CTL therapy for primary ATL has shown promise leading to prolongation of survival time [25,26]. Very recently, promising results from phase I clinical trials against ATL using Tax peptide-pulsed DC-based vaccination have been reported at the 16th International Conference on Human Retrovirology, June 26–30, 2013, Montreal, Canada (Suehiro et al., abstract no. O1-2251). A significant reduction in proviral loads and size of lymph nodes with no severe side effects were observed in patients confirming a therapeutic effect. Tax oligopeptide, particulary Tax(11–19), as a dominant CTL epitope can be harnessed to induce antitumor immunity, suggesting that it could be a suitable candidate for a DC-based vaccine against HTLV-1-induced ATL [27,28]. Previously, we have shown that following exposure to Tax, murine and human DCs undergo activation and maturation, exhibit changes in activation markers, surface phenotype, and secretion of cytokines/chemokines, leading to allogenic and Tax-specific immune responses [29–33]. We concluded that both Tax protein and its epitope (11–19) are potent enough to drive efficient antigen-specific CTL responses in naïve PBLs from normal donors as well as in HLA-A2.1 transgenic mice [34]. Hence, the promise of ATL therapy lies in reinstating a patient's anti-HTLV-1 CTL response by developing DCs pulsed with the immunodominant epitope Tax(11–19) to facilitate recognition. In this respect, we [34] and others [35] have previously reported that Tax-pulsed DCs make highly potent immunostimulators; however, in vivo evidence has not been yet reported for the immunogenicity of the naturally identified HLA-A*0201-restricted epitope 11–19 and the impact of DCs in this process.

DC priming in early stages of HTLV-1 infection has also been shown to be important for controlling disease progression [36]. To prove this, we had previously used a CD11c-diphtheria toxin receptor (DTR) transgenic mouse model [37,38] that permits conditional transient depletion of CD11c+ DCs. Infection of these mice was performed with chimeric HTLV-1 virus wherein the envelope gene of HTLV-1 was replaced with that of the ecotropic Moloney murine leukemia virus (Mo-MLV) for better fusion of envelope with murine cells [39] and better induction of humoral and cellular immune responses [40]. Upon both cell-free and cell-associated infection, we witnessed an increase and decrease in proviral load respectively, in both CD4+ and non CD4+ T-cell fractions and significant reduction in IFN-γ response in the CD8+ T cells. These results proved the importance of DCs in controlling cell-free virus and pointed toward the involvement of DCs in cell-associated infection. Here we wish to provide direct in vivo evidence for the importance of DCs with respect to in vivo immunogenicity of the HLA-A2-restricted Tax (11–19) epitope, which is immunodominant in both carriers of HTLV-1 and patients with the disease. To this end, C57BL/6-Tg (HLA-A2.1)1Enge/J male mice that express significant quantities of the human class I MHC Ag HLA-A2.1 were crossed with B6.FVB-Tg Itgax-DTR/EGFP 57Lan/J (CD11c-DTR) mice that express the simian DTR-enhanced green fluorescent protein (EGFP) under the control of the Itgax (or CD11c) promoter. The CD8+ T-cell immune response in the newly hybrid HLA-A2.1/DTR transgenic mice were tested for both the naïve and a restimulation responses against Tax(11–19) antigen delivered with tetanus helper peptide (THP) in the absence or presence of adjuvant IFA (incomplete Freund's adjuvant; used as one experimental approach for immunogen delivery). In vivo depletion of CD11c+ DCs abrogated CD8+ T-cell responses in the absence and presence of adjuvant, thereby implicating the initial priming of DCs with Tax(11–19) peptide in the successful generation of an efficient antigen-specific response. Depletion of DCs coincided with a much higher level of IL-6 in sera of mice that had not received IFA. This is the first report to our knowledge wherein a transgenic hybrid mouse model carrying HLA-A2.1 and DTR transgenes together was utilized for studying the CD8+ T-cell immune response directed against a dominant HTLV-1 Tax(11–19) antigen. This in vivo model system could also facilitate future studies of DC-mediated HLA-A2-restricted antigen-specific immune responses.

2. Results

2.1. Generation of novel transgenic hybrid mice containing HLA-A2.1/DTR transgenes

Transgenic hybrid mice generated from an intercross between HLA-A2.1 and DTR transgenic mice were all healthy and viable. The presence of the HLA-A2.1 transgene (111 bp) was observed along with a control gene fragment (MGSCv37; Mouse Genome Sequencing Consortium for The *Mus musculus* strain C57BL/6J build 37) of 200 bp and the DTR gene was detected by amplifying a 625-bp gene fragment (Fig. 1A, upper panel). The HLA-A2.1 transgene was found in 100% of the F1 hybrid progeny and the DTR transgene was shown to be present in 49% of the hybrid progeny when 66 pups of the F1 generation were analyzed (52% in females and 46% in males) (Fig. 1A, lower panel). These results were expected given the homozygous nature of HLA2.1 mice and the hemizygous nature of CD11c-DTR mice. Only double-positive mice were utilized in subsequent experiments.

2.2. Depletion of CD11c+ DCs in HLA-A2.1/DTR mice by the administration of diphtheria toxin

The dose, timing, and route of DT administration were implemented as previously described [36]. In vivo depletion of conventional murine splenic DCs from hybrid mice were confirmed by assessing the frequency of CD8α+/CD11c+ cells before and after DT treatment (Fig. 1B). As expected, most of the splenic DC population was ablated within 24 h of DT injection and was reduced to an average of 1.3% as compared with 5.5% of total CD8+ splenocytes in the non-DT control group, as previously observed [36]. Similarly, the reduction in DC frequency slowly recovered by day 5 (data not shown), making it essential to complete the subsequent immunization studies within a 5-day interval. Since studies suggest the expression on CD11c on activated CD8T cells [41,42], we also determined the frequencies of CD8α+ T cells. It was found that DT administration did not affect either the frequency of CD8α+ T cells from which the CD11c+ cells were gated or CD4+ T cells (Supplementary Fig. 1) which were also looked at.

2.3. Depletion of DCs abrogated the immunogenicity of Tax(11–19) epitope

In previous studies, we demonstrated the immunogenicity of Tax(11–19) epitope both in vitro and in vivo in line HHD II mice (expressing chimeric human and mouse HLA-A2.1 heavy chain linked to human β2-microglobulin) [34]. Here the impact of DC depletion on this process was examined in the newly hybrid strain. Levels of CFSE were first assessed on days 1 and 12 from splenocytes of control, nonimmunized mice stimulated in vitro with mitogen Con A (positive control), Tax(11–19) peptide, BMDCs,

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