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Suppression of inducible CD4 regulatory cells by MHC class I-restricted human tumor epitope specific TCR engineered multifunctional CD4 T cells

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ABSTRACT

Regulatory T cells (Treg) can interfere with the generation and function of anti-tumor immune effectors. Accordingly, ways that could block Treg function would be useful in cancer immunotherapy. We have previously shown that incorporation of CD4+CD25-ve T cells in an in vitro cytolytic T lymphocyte (CTL) generation assay leads to generation of induced regulatory T cells (iTregs), and that these iTreg block the generation of productive CTL response (Chattopadhyay et al., 2006). We here show that human CD4 T cells engineered to express MHC class I-restricted human melanoma associated epitope, MART-1_{27-35}, specific T cell receptor (TCR), that can simultaneously exhibit helper as well as cytolytic effector functions (Chhabra et al., 2008, Ray et al., 2010), can interfere with the generation of inducible Treg, block iTreg-mediated suppression, and allow the activation and expansion of MART-1_{27-35} specific CTL responses, in vitro. We also show that mitigation of Treg generation by TCR engineered CD4 T cells is not mediated by a soluble factor and may involve "licensing/conditioning" of the dendritic cells (DC). Our data offer novel insights on the biology of MHC class I restricted TCReng CD4 T cells and have translational implications.

1. Introduction

Regulatory T cells (Treg) play important roles in preventing autoimmunity and in the regulation of immune responses by controlling continued and uncontrolled inflammation [1–3]. CD4+ Treg have been shown to have protective roles in autoimmune type 1 diabetes, in asthama, as well as in controlling inflammatory bowel disease [1,3,4]. However, in context to anti-tumor immunity, regulatory T cells serve as a limiting factor. While thymus derived CD4+CD25+FoxP3+ natural Tregs (nTregs) were initially considered to be the major type of Tregs [2,5,6], it is now well established that an inducible form of Tregs (iTreg) can arise from CD4+CD25-ve precursors in the periphery [6,7]. Both nTreg and iTreg have been found to have suppressive properties in antitumor immunity [8–10]. However, in an in vitro cytolytic T lymphocyte (CTL) generation assay, incorporation of CD4+CD25-ve T cells in an in vitro cytolytic T lymphocyte (CTL) generation assay leads to generation of induced regulatory T cells (iTregs), and that these iTreg function far more effectively regulators than the nTregs in blocking the generation of a productive CTL response [11].

Given that Tregs can suppress CTL function in antitumor immunity, a variety of approaches have been, and are being, tested in tumor immunity, at the bench and in the clinics. In this context, Caretto et al. have shown that the Th1 type responses can inhibit the generation of peripheral Tregs in a transgenic mouse model [12]. Utilizing human melanoma associated antigenic epitope, MART-1_{27-35}, specific transgenic TCR, we have recently shown that human CD4 T cells can be programmed to exhibit a Th1 type effector functions, that can not only facilitate help towards the expansion of CD8+CTL response generation, but also exhibit MHC class I restricted cytolytic function of their own [13,14]. Given that help and suppression are two polar opposites in the functional spectrum, we here examined whether the TCReng CD4 T cells can mitigate the regulatory T cell mediated suppression in our melanoma epitope specific CTL generation system, in vitro. We show here that the TCReng CD4 T cells can indeed facilitate CD8+CTL response generation by mitigating iTreg-mediated suppression. We also show that the interference of iTreg generation by the TCReng Th1 type CD4 T cells is not mediated by a soluble factor and that in co-culture with dendritic cells (DC), they exhibit activities

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analogous to "licensing/conditioning" antigen presenting cells [15–19]. Our data offer novel insights into the biology of MHC class I restricted tumor epitope specific TCR engineered CD4 T cells and suggest that these customized immune effectors have translational potentials.

2. Materials and methods

2.1. Study population, cell lines, culture medium and reagents

The study population included HLA-A2-positive healthy donors harboring precursors for MART-1_{27-35} epitope specific CD8+ T cells in their peripheral blood. Participation in the study was strictly voluntary and all the donors were enrolled with informed consents, in accord with the Institutional Review Board (IRB) guidelines. Cell culture medium, cytokines and growth supplements used to culture the human peripheral blood derived T cells, monocytes, and differentiation of monocytes into dendritic cells (DC) were purchased and used as described before [13,14]. T2-A2 cell line used to present peptide epitopes to the antigen specific T cells has also been described before [13,14]. Antibodies and tetramer reagents to track T cells and antigen specific CTL were purchased from BD Pharmingen (USA), eBioScience (Affymetric, USA) Beckman Coulter (USA), and R & D Systems Inc. (USA), and have also been described before [13,14].

2.2. Transduction of CD4 T cells with transgenic TCR

CD4+CD25- and CD8+ T cells were purified from peripheral blood derived leukocytes (PBL) of HLA-A2+ve donors utilizing dynal magnetic bead isolation system (Invitrogen, USA0. For transduction, CD4+CD25- and CD8+ T cells were activated with plate-bound anti-CD3 and CD28 antibodies in 100 units/ml IL-2 containing IMDM medium, and 2–3 day activated cells were transduced with MART-1_{27-35} (M1) epitope specific transgenic TCR, as described before [14,20]. Following transduction, cells were maintained in 1000 units/ml IL-15. Un-engineered CD4 T cells were kept in culture in IMDM medium containing IL-15 cytokine and used as controls. Transduction efficiency was quantified by tetramer staining by FACS. TCR transduced CD4 and CD8 T cell populations were expanded by co-culturing them with the M1 peptide pulsed mature DC, according to our published methods [13,14].

2.3. In-vitro CTL generation assay

Human melanoma associated antigenic epitope, MART-1_{27-35}, specific CD8+ natural CTL were generated by co-culturing peripheral blood derived CD8+ T cells (with at least 1×10^6 CD8+ T cells per set) with mature dendritic cells (mDC), as described previously [13,14,21]. In brief, blood derived immature DC (iDC) were matured by overnight treatment with LPS and IFN-γ and co-cultured with magnetic bead (Dynabeads, Invitrogen, USA) purified human peripheral blood derived CD8+ve T cells. To examine the effect of engineered CD4 T cells on Treg mediated suppression, TCR engineered CD4 T cells or the control activated CD4 T cells were added to the DC cultures 2 h before adding the CD8 T cells with/without natural and the induced Treg cells (CD4: CD8 ratio 1:10). As expected in human subjects, donor to donor variability was observed in generating induced regulatory T cells in DC co-cultures. Donors exhibiting iReg effect were chosen for our further studies to establish the effect of TCReng CD4 T cells. Double chamber experiments were also done in similar fashion except that the DC were plated in tissue culture well for DC-CD8 T cell co-culture, as well as on the floor of the trans well chamber (BD Biosciences, USA) with TCReng CD4 T cells or the control CD4 T cells. Co-cultures were followed for 7–10 days before quantifying the expansion of MART-1_{27-35} epitope specific CTL precursors by FACS analysis following MART-1_{27-35} epitope specific tetramer staining. For functional characterization of CD8+CTL generated in the presence of TCReng CD4 T cells, CD8 T cells were purified by dynal magnetic bead isolation method (Invitrogen, USA), and effector function was characterized by measuring cytokine release upon cognate antigen encounter by ELISA.

2.4. Effector function characterization of transgenic TCR engineered CD4 T cells

Function of TCReng CD4 T cells was characterized by examining their effector cytokine production profile following culturing them with T2-A2 cells, alone or T2-A2 cells pulsed with control peptide, MAGE-$3_{271-279}$, or the cognate peptide, MART-1_{27-35}, for 16 h, as described before [13,14]. In brief, following 16 h co-culture, supernatants of the co-cultures were tested for specific cytokines, by ELISA, utilizing R&D systems duoset ELISA kits, as per manufacturer's recommendations.

2.5. DC Conditioning and functional analysis of "conditioned" DC by ELISA

Human peripheral blood derived immature dendritic cells (iDC) were pulsed with MART-1_{27-35} peptide and co-cultured with either control un-engineered or MART-1_{27-35} epitope specific TCR engineered CD4 T cells (Effector: Target = 1:10). DC conditioning was measured by FACS-mediated surface phenotype and functional analyses. Cytokines released from "conditioned" DC were measured by sandwich ELISA kit (R&D Systems, USA) according to the manufacturer's instruction. As control, blood derived iDC were matured with LPS and IFN-γ, according to our published methods [13,14,21], and surface phenotype of iDC and mature DC (mDC) was examined by FACS.

2.6. Statistical analysis

Functional analysis of effector T cells was done in experiments set up in triplicates, and statistical analysis of the data was done by standard methods available in Microsoft Excel, such as mean and standard deviation calculations. The p-values were calculated by *t*-test using GraphPad Prism software.

3. Results

3.1. Fresh CD4+/CD25- T cells interfere with CTL expansion while epitope specific TCReng CD4 T cells provide "help" in CTL response generation

Fig. 1A shows generation of TCReng CD4 T cells by engineering peripheral blood derived CD4+CD25- T cells with a MART-1_{27-35} epitope specific transgenic TCR, and their Th1 biased effector cytokine production profile, in accord with our published results [13,14,20]. As shown, we can generate 64.3% MART-1_{27-35} epitope specific tetramer positive cells upon transduction and following one antigen exposure to the cognate peptide on autologous mature DC, we can generate a near homogenous antigen specific CD4+ T cell population.

In order to examine whether these TCReng CD4 T cells could mitigate the generation of iTreg and facilitate the activation and expansion of epitope specific cytolytic CD8 T lymphocytes in our tumor epitope specific CTL generation assay, in vitro [21]; we first recapitulated the two remarkably opposite activities exhibited by the autologous CD4+CD25- T cells in the CTL generation culture,