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Human CD8⁺ T Regulatory cells with low avidity TcR specific for minor Histocompatibility antigens

William J. Burlingham¹ and Els Goulmy²

¹University of Wisconsin, Dept. of Surgery, 600 Highland Ave, Madison, WI, burlingham@surgey.wisc.edu

²Leiden University Medical Centre, Dept. of Immunohematologie, Leiden, the Netherlands

Abstract

Maternal / fetal microchimerism resulting from cell exchanges during pregnancy constitutes a reservoir of persisting alloantigen in mother and adult offspring. These alloantigens induce Minor Histocompatibility (H) antigen-specific immune responses in both mother and her offspring, including CD8⁺ T regulatory [T_R] cells with low T cell receptor [TcR] binding to MHC tetramers. Although they bind cognate MHC/peptide relatively poorly, these CD8 T_R nonetheless inhibit high avidity, tetramer-bright CD8 T effector [T_E] responding to the same minor H antigen through induction of immunosuppressive DC products. In this review article we explore the mechanisms of such 'low avidity' CD8 T_R -dependent suppression and discuss their role in naturally acquired tolerance to familial minor H antigens encountered during gestation and in parous females. We discuss the implications of our findings for chronic/persisting viral infections, residual tumor burden after cancer treatment and immunotherapy, and renal allograft tolerance.

Introduction

CD8 T cells bearing receptors [TcRαβ] with high avidity for cognate peptide/MHC set the tone for a broad spectrum response to pathogens. Strong intracellular signals generated at the immune synapse activate dendritic cells [DC] to upregulate ICAM-1 and thereby enable a single DC to recruit many intermediate and low avidity T cells into the host response [1]. Signals generated within the T cell also result in mobilization of CTLA4 to the immune synapse, dampening further signaling while allowing low avidity T cells with weaker signal generation [and less CTLA4 mobilization to the synapse] a way to overcome their competitive disadvantage[2]. As the primary immune response wanes during pathogen elimination and effector T cell apoptosis, both low and high avidity CD8 T memory cells can form and be maintained via homeostatic proliferation, ensuring a broad TcR repertoire in response to secondary challenge. Innate immunity also helps to protect a broad adaptive T cell repertoire by providing a cytokine milieu [e.g. IL-1β, IL-6] during acute infection that imparts resistance to inhibitory effects of T regulatory cells [T_R], making sure that the T_E are not led astray into becoming T_R themselves.

Under certain circumstances, however, the source of foreign antigen is not eliminated. For example, adaptive CD4 T_R that arise during *Leishmania* infection can effectively prevent

Correspondence to: William J. Burlingham.

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parasite elimination[3]. Similar mechanisms may operate to prevent ocular immunopathology in herpes simplex virus infections [4]. Thus T_R may protect a reservoir of persisting antigen, able to chronically stimulate host T cells, while blocking collateral damage to tissues harboring the pathogen. In some cases, e.g. CD4 T cells responding to Leishmania antigens, chronic persisting infection helps to maintain a more 'vigilant' T memory pool[3]. In CD8 T cell responses to viral infection, chronic persisting infection may alter the memory pool in a different way. Specifically, while antigen-independent, IL-7-driven CD8 T memory responses arise after viral clearance in acute infection models and provide robust immune memory, CD8 T cells in the chronically infected host become much more antigen-dependent for survival and lack expression of IL7 receptor [5] [6]. Chronic persisting alloantigen that arises from maternal/fetal cell exchange during pregnancy may also give rise to antigen-dependent CD8 T memory cells; thus, microchimerism and allospecific CD8 T_R development may be interdependent phenomena [see below].

Certain pathogens may take advantage of T_R - T_E homeostasis by disrupting the fine tuning of the balanced host immune response to infection. A study by Garba et al.[7] showed that HIV antigens can induce TGF β 1-producing, immunoregulatory CD8+ T cells in chronically - Infected individuals. PBMC from healthy individuals were induced to express IFN γ in their CD8 T cells by exposure to a vaccinia virus vector. When antigenic HIV peptides were co-expressed with vaccinia antigens, their CD8 T cells made IFN γ equally well. However the same vaccinia/HIV stimulus induced CD8 T cells from HIV-infected patients to produce cell surface TGF β 1, markedly suppressing the strong IFN γ production that was observed in response to vaccinia alone. This study established for the first time that chronic persisting viruses such as HIV can subvert host immune responses by inducing a 'non-classical' TGF β 1-producing CD8 T_R .

The tetramer-dim minor H antigen-specific CD8 T_R

In attempt to find a possible linkage between microchimerism and tolerance to an HLA-matched, minor H antigen mismatched renal transplant, the role of the hematopoietic system-restricted minor H antigen HA-1 in renal allograft tolerance was explored[8]. The HA-1 locus encodes two alleles, i.e. HA-1H and HA-1R. HA-1H is the immunogenic allele presented in the context of HLA-A2 and the individual expressing it is referred to as HA-1⁺. The HA-1R allele is the "null" allele and the R/R homozygous individual is referred to as HA-1^{neg}. We analyzed three patients who had each received HLA-matched but HA-1 mismatched renal transplant, one of whom had discontinued immunosuppression over 30 years previously while sustaining normal kidney function[8]. The PBMC of the three HA-1^{neg} patients were injected into the footpads of CB17/scid mice to measure their DTH reactions in response to a donor cell lysate or HA-1 peptide. All three patients showed TGF- β and/or IL-10 regulated DTH responses to HA-1 peptide and to the composite pool of donor [minor H] antigens. By analyzing HA-1 specific CD8+ T cell tetramer staining intensities, we observed two distinct minor H antigen HA-1 specific T cell subsets based on their binding intensities of the HA-1 bearing tetramer. The tetramer dimly staining population of HA-1 specific CD8+ T cells showed characteristics of a T_R cell, producing IL-10 and TGF- β . The tetramer brightly staining population of HA-1 specific CD8+ T cells showed characteristics of a T_E cell, mediating a strong DTH response and producing IFN- γ . Suppression of the T_E cell function by T_R cells was TGF- β , IL-10, and CTLA-4 dependent. Interestingly, HA-1⁺ microchimerism was detected in all three renal allograft recipients, and was primarily present in the T cell and/or dendritic cell fractions[8,9]. These data demonstrated for the first time a 3-cell compartment including minor H antigen-specific CD8+ T_R cells, minor H antigen-specific CD8+ T_E cells, and minor H antigen-expressing microchimerism in recipients of renal allografts. It should be noted that the one patient who had successfully discontinued all immunosuppressive therapy had a history of familial minor H antigen exposure. She had delivered an HA-1+ child 18 years

before transplant, and her own mother was also HA-1+. Therefore, the observed Mc in this patient could have resulted from the donor transplant (donor Mc), the patient's child (fetal Mc), or her mother (maternal Mc). This possibility leads to the hypothesis that maternal/fetal microchimerism, a reservoir of familial MHC and minor H alloantigens, were present prior to transplantation, and predisposed the patient to tolerance of an HLA-matched, minor H antigen-mismatched renal transplant.

The normal adult harbors minor H antigen specific, tetramer-dim CD8 T_R

To test whether healthy adults harbor minor H antigen CD8 T_R cells, we recently performed a family analysis. Hereto, PMBCs of healthy blood donors with minor H antigen disparate family members, thus mothers and their offspring, were studied for the putative presence of minor H alloantigen specific T_R cells. Indeed, minor H alloantigen-specific T_R were present in the PBMCs of healthy men and women. These Treg arose across various minor H antigen barriers and expressed CTLA-4 on their cell surface, a molecule that was shown to be involved in their suppressive function[10]. Our observation that T_R reside within the tetramer^{dim} staining fraction confirms two earlier studies describing the phenomenon of reduced HLA/peptide multimer binding by T_R^{15,18}. However, a tetramer^{dim} staining phenotype is not exclusively associated with a regulatory function, since low-avidity CTL can also be expanded from tetramer^{dim} populations[8]. This is consistent with the assumption that low avidity CD8 T cells may have dual functions. For example, they provide an important component of a healthy immune response to pathogens, carefully nurtured and brought into the adaptive immune response by DC that have been activated by high avidity T cells under pro-inflammatory conditions. Yet they also can function as key players in the dampening of the host immune response under non-inflammatory conditions. How low avidity CD8 T cells are recruited into the T_R pathway is still unclear. The complex phenomenon of anterior chamber-associated immune deviation, which is CD1d/NKT-dependent, and ultimately results in the induction of CD8 T_R. The latter remains the best studied example of minor H -specific CD8 T_R induction arising from antigen introduced in an immune privileged environment[11]. The liver is another known immune privileged site. It is possible that microchimerism in the liver parenchyma arising from engraftment of maternal or fetal stem cells may feed antigen into the host immune system in a tolerogenic manner. Recent analysis of a mouse model of maternally-induced transplantation tolerance using sensitive PCR techniques for detection of maternal cells, indicates that maternal microchimerism is strongly correlated with the induction of TGFβ-producing adaptive T_R [12]. Interestingly, the liver was one of the major sites for maternal Mc, while the spleen was not. Fortunately, both in mouse and human systems the peripheral blood appears to harbor a sufficient level of microchimerism for cell subset analysis [12],[10]. Relying on the PBMC of healthy adults, our preliminary data indicate an association between microchimerism detectable only in lineages with a relatively short half life [PMNs, rbc], and the presence of tetramer^{bright} CD8 CTL coupled with lack of regulatory function in TV-DTH assays vis a vis familial minor H antigen. On the contrary, in healthy adults with predominantly tetramer^{dim} CD8 T cells, we observed minor H antigen-specific regulatory function in TV-DTH, and microchimerism in long-lived lineages, and particularly in those with both HLA class I & class II expression. This may point to better engraftment of maternal or fetal stem cells in the latter individuals, giving rise to multilineage microchimerism. A similar finding has recently been reported in mice with microchimerism in MHC class II+ and class I+ lineages. These transplant recipients displayed a robust form of allotolerance, as compared with mice with T cell microchimerism only, which had split tolerance [13].

Mechanisms of T_R programming via low avidity TcR-pMHC interaction

Over 10 years ago, Allen and colleagues observed that altered peptide ligands can induce a qualitatively different pattern of signal transduction events in T cells than does any

concentration of the native ligand[14]. Such observations imply that several signaling “modules” are directly linked to the TCR/CD3 complex and thus can be dissected as a direct result of the nature of the ligand bound. This leads to speculation that one such ‘module’ is the complex of responses found in suppressive T cells, requiring a partial signal generated through the TcR/CD3 complex. Consequently, CD8 T cells with low avidity TcR specific for pMHC may have a selective advantage as suppressive T cells, favoring an over-representation of minor H antigen -specific T_R in this subset. Such an avidity-based model of T cell suppression has been proposed to account for the opposite functional states of CD1d-restricted NK-T cells which express an invariant TcR α chain [iNK-T]. Specifically, iNK-T responses to the strong agonist glycolipid antigen *agal*-cer feature Ca⁺⁺ flux and production of IFN γ , whereas stimulation by weak agonist ‘self’ glycolipid antigens results in partial signaling and IL-4/IL-13 production[15]. Our understanding of the differences between CD8 T cells with high and low avidity TcR specific for a given minor H antigen is still incomplete. We previously demonstrated that the intensity (^{bright} or ^{dim}) of tetramer staining by *in vitro* generated minor H antigen specific cytotoxic T cell clones strongly correlates with their capability of recognizing minor H antigen^{pos} target cells [16]. We therefore designated tetramer^{bright} staining T cells as high avidity T cells recognizing natural ligand expressing target cells and tetramer^{dim} staining T cells as low avidity T cells recognizing only exogenous peptide pulsed target cells. However, this classification based on CTL activity does not exclude a role for tetramer^{dim} staining T_R cells in the functional recognition of natural ligand on donor target cells. This is because the function of tetramer^{dim} staining, minor H antigen-specific T_R cells appears to be primarily mediated through induction of immunosuppressive products in dendritic cells(DC), including donor DC expressing naturally processed pMHC ligands [17]. The co-dependence of suppression on CD8 T_R and DC has been noted previously[18].

Diminished CD8 expression and altered TcR $\alpha\beta$ as alternative pathways to reduced TcR signalling in CD8 T_R

Studies underway in the laboratory of one of us[E.G.] already suggest that the TcR $\alpha\beta$ gene repertoire of the tetramer^{dim} CD8 T_R may be broader than that of tetramer^{bright} CD8 T_E specific for HA-1 [19][M Dierselhuis et al., in prep]. Thus there may be altered selection of TcR $\alpha\beta$ in these 2 subsets, leading to differential TcR avidity. The general principle of low avidity CD8 T suppressor cells regulating conventional high avidity CD8⁺ T_E cells has recently been confirmed in an animal model of male-to-female H-Y specific skin graft tolerance induced by incomplete antigen stimulation [signal 1 only] *in vivo* [20]. However, unlike our human studies in which HY and HA-1 tetramer^{bright vs. dim} analysis was done only on CD8^{bright} T cells, in the mouse model a diminution in surface expression of CD8 α and CD8 β on HY-specific T cells was found to be the major route to generation of a low avidity TcR/CD8 signaling complex [21,22]. Here CD8^{dim} staining HY-specific Treg were found to regulate CD8^{bright} CTL via TGF β production [20]. This result suggests that incomplete T cell receptor signaling may be a common feature of CD8 T reg function, regardless of the route [altered TcR $\alpha\beta$ selection vs. diminished CD8 $\alpha\beta$ expression] taken by the T cell. In the mouse, loss of CD8 expression may be induced by IL-4[23]. CD8 loss may also go to completion, as in the phenomenon of the so-called ‘double-negative’ T_R cell first described in the 2c TcR transgenic mouse system[24]. There is evidence that such double-negative T cells can produce latent TGF β in the heart allografts of tolerant mice [Torrealba, J. & Zhang, L. personal communication].

Summary and Conclusions

The findings of ‘low avidity’ CD8 T regulatory cells in minor H antigen-specific transplant tolerance and in natural tolerance to familial antigens acquired during ontogeny and/or pregnancy have broader implications for immunologic tolerance strategies and for tumor vaccine design. In the case of transplantation of solid organs and bone marrow from living

donors, the pre-existence of familial minor H antigen-specific, tetramer^{dim} T_R in healthy individuals may be an untapped resource waiting to be exploited for tolerance induction trials in humans, particularly in patients with haploidentical and HLA-identical living donors [25, 26].

In tumor immunotherapy, a major obstacle is the ability of the tumor to subvert effector T cell responses. One way this happens is that low avidity CD8 T cells accumulate among the tumor-infiltrating lymphocytes[TIL], leading to diminished effector function[27]. CD8+ TIL with reduced avidity for pMHC may be readily recruited into the ranks of tumor antigen-specific T suppressors, rather than into a CTL differentiation pathway. Strategies such as CTLA4 blockade combined with tumor vaccination [2] may restore effector function in the TILs by shifting the balance in favor of tetramer^{bright} CD8 T effector cells. Furthermore, by relieving suppression imposed by CTLA4, a characteristic feature of the tetramer^{dim} T_R, the uncovering of previously latent CTL responses can enable the discovery of novel targets for tumor immunotherapy [28].

In conclusion, as we continue to learn more about tetramer^{dim} CD8 T regulatory cells and their role in tolerance and immune homeostasis, our studies will undoubtedly illuminate important features of regulatory T cells in general, features that can potentially be exploited for therapy of a variety of human diseases.

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