Published in final edited form as:
Curr Opin Immunol. 2008 June ; 20(3): 327–331. doi:10.1016/j.coi.2008.02.003.

EMERGING CONCEPTS IN CD8+ T REGULATORY CELLS

Jerry Y. Niederkorn
Department of Ophthalmology, University of Texas Southwestern Medical Center, Dallas, Texas 75390 U.S.A.

Summary

CD8$^+$ T regs are elicited by unique antigen presenting cells during viral infections, by manipulation of co-stimulatory molecules, or in the development of tumors. CD8$^+$ T regs display antigen-specificity, which is most exquisitely manifested by the HLA-E-restricted cytolytic CD8$^+$ T regs in MS. There is evidence that some CD8$^+$ T regs also express organ specificity. In many cases, IFN-γ is required for either the induction or expression of CD8$^+$ T regs. CD8$^+$ T regs can produce suppression directly by killing immune cells or indirectly by co-opting other cells to elaborate end-stage suppressive molecules such as TGF-β, IL-10, and indoleamine dioxygenase (IDO).

Introduction

The seminal findings of Sakaguchi and co-workers resurrected the concept of suppressor cells and demonstrated the role of CD4$^+$CD25$^+$ T cells in self-tolerance[1]. Legitimization of suppressor cells was marked by their new euphemistic moniker, T regulatory cells (T regs). The past decade has witnessed an explosion in the number of studies on both natural and induced CD4$^+$CD25$^+$ T regs, while research on CD8$^+$ T regs has received considerably less attention. In spite of this, significant new insights have come to light and indicate that CD8$^+$ T regs play a unique role in restoring immune homeostasis and in maintaining immune privileged sites.

Induction of CD8+ T regs via Co-Stimulatory Molecule Interactions

CD4$^+$CD25$^+$ T regs can arise naturally in the thymus or can be induced by a variety of manipulations. By contrast, CD8$^+$ T regs do not normally occur naturally, but are induced under a variety of conditions including manipulation of co-stimulatory molecule interactions [2–4], antigen processing by unique antigen presenting cells (APC)[5,6], during viral infections [7], or in the development of some tumors[8].

The co-stimulatory molecule CD137 (4-1BB) is important for immunity against tumors and viruses, yet it also inhibits experimental autoimmune diseases[3]. Myers and co-workers recently showed that immunization with OVA in combination with polyI:C and anti-4-1BB induced the generation of CD8$^+$ T regs that profoundly inhibited CD4$^+$ T cells[3]**. The anti-4-1BB-induced suppression was mediated by CD8$^+$ T regs and required the presence of IFN-γ, yet IFN-γ alone did not mediate suppression. CD8$^+$ T regs did not function unless they produced and responded to IFN-γ. That is, anti-4-1BBinduced CD8$^+$ T regs did not develop in IFN-γR $^{-/-}$ mice. Binding of IFN-γ to the CD8$^+$ T regs stimulated the production of TGF-

Corresponding Author: Jerry Y. Niederkorn (jerry.niederkorn@utsouthwestern.edu).

β, which acted as the end stage suppressive molecule. Moreover, addition of neutralizing anti-TGF-β reversed the suppressive effects of $CD8^+$ T regs. Thus, engagement of the co-stimulatory molecule 4-1BB generates $CD8^+$ T regs that both produce and respond to IFN-γ, which in turn, induces the elaboration of TGF-β, which suppresses antigen-specific proliferative responses of $CD4^+$ T cells.

A recent study demonstrated that blockade of the co-stimulatory molecule, CD40, promoted the indefinite survival of heart allografts in rats that was mediated by $CD8^+$ T regs[9]**. Graft survival could be adoptively transferred with spleen cells through four generations of hosts. This "infectious tolerance" was entirely dependent on $CD8^+$ T cells and IFN-γ, as treatment with either anti-CD8 antibody or neutralizing anti-IFN-γ antibody resulted in allograft rejection. Allografts contained an abundance of IFN-γ, which is a major stimulus for the induction and expression of indoleamine dioxygenase (IDO). IDO is a potent inhibitor of tryptophan metabolism and it is widely recognized that tryptophan deprivation leads to T cell apoptosis. Blockade of IDO with 1-methyl tryptophan resulted in rapid graft rejection in tolerized rats. Immunohistochemical analysis revealed that IDO was expressed primarily in the vascular endothelial cells of the heart allografts. This demonstrates for the first time, the generation of a novel $CD8^+$ T reg population that mediates infectious tolerance through at least 4 generations and promotes allograft survival by co-opting cells within the allograft to produce the potent immunosuppressive molecule IDO. This in turn creates an *ad hoc* immune privileged site.

Antigens Introduced into an Immune Privileged Site Induce $CD8^+$ T regs

One of the more important factors that contributes to ocular immune privilege is the unique immune deviation that is elicited when antigens are introduced into the anterior chamber (AC) [10,11]. Anterior chamber-associated immune deviation (ACAID) culminates in the appearance of $CD8^+$ T regs that suppress Th1 immune responses, such as delayed-type hypersensitivity (DTH), and deviates antibody responses from complement-fixing isotypes to non-complement-fixing isotypes. $F4/80^+$ ocular APC capture antigens introduced into the AC and then migrate to the spleen where they initiate a series of cellular interactions involving NKT cell emigrants from the thymus[12,13], B cells[5,6,14] $CD4^+$ T cells[15,16], $CD8^+$ T cells[17], and γδ T cells[18,19]. The process begins when ocular APC release antigenic fragments, which are captured by antigen-specific B cell receptors on splenic B cells. After internalizing and processing the cognate antigen, B cells proliferate thereby expanding the numbers of antigen-specific splenic B cells that are available for capturing and presenting antigen to T cells[5,6,20]. B cells utilize both MHC class I and class II molecules to present peptides to $CD8^+$ and $CD4^+$ T cells respectively[5]*. Following antigen presentation by B cells, the T cells differentiate into $CD4^+$ T regs and $CD8^+$ T regs.

Generation of ACAID $CD8^+$ T regs also requires the participation of splenic γδ T cells[18, 19]. Although γδ T cells can act as APC in other settings[21], they do not participate in antigen presentation in ACAID, and instead they serve as important sources of IL-10 within the splenic milieu[22]. γδ T cell-derived IL-10 is required for the induction of ACAID $CD8^+$ T regs, as reconstitution of γδ $TCR^{-/-}$ mice with γδ T cells from IL-10 $^{-/-}$ mice fails to restore ACAID $CD8^+$ T regs, while reconstitution with γδ T cells from IL-10 competent mice does.

NKT cells also participate in the generation of ACAID. Within the spleen, CD-1d-reactive NKT cells produce RANTES, which recruits precursor $CD8^+$ T regs and additional eye-derived $F4/80^+$ APC to the spleen [23]. NKT cells produce IL-10, a crucial cytokine needed for the generation of ACAID $CD8^+$T regs[24]. NKT cells also produce urokinase-type plasminogen activator (uPA), which contributes to the generation of $CD8^+$T regs by binding to the uPA receptor on on $F4/80^+$ APC within the spleen and converting latent TGF-β into its active form.

[25]**. Active TGF-β, along with NKT cell-derived IL-10, promotes the generation of $CD8^+$ T regs.

ACAID $CD8^+$ T regs inhibit DTH by previously sensitized $CD4^+$ T cells by a mechanism that requires IFN-γ[26]. Neither ACAID $CD8^+$ T regs from IFN-$\gamma R^{-/-}$ mice nor IFN-γ alone can inhibit DTH. However, IFN-γ acts on $CD8^+$ T regs induced in normal mice and "licenses" them to express their suppressive function *in situ*. The mode of suppression by $CD8^+$ T regs remains to be elucidated, but neither perforin-mediated cytolysis nor FasL-induced apoptosis is necessary[26]*.

$CD8^+$ T regs in Multiple Sclerosis

Investigations on T regs in multiple sclerosis (MS) have been largely limited to the roles of $CD4^+$ T regs, however recent studies have demonstrated the participation of $CD8^+$ T regs [27,28]. $CD8^+CD122^+$ Tregs spontaneously arise in the mouse experimental autoimmune encephalomyelitis (EAE) model of MS. *In vivo* depletion of $CD8^+CD122^+$ cells exacerbates EAE in mice, while adoptive transfer of $CD8^+CD122^+$cells from naïve donors to recipients at the height of clinical disease mitigates EAE [29]. Correale and Villa detected the presence of spontaneously occurring $CD8^+$ T regs in the peripheral blood of MS patients[27]*. $CD8^+$ T regs isolated from MS patients lysed myelin-reactive $CD4^+$ T cells by a cytolytic process that was granule-mediated and restricted by the non-classical MHC Class Ib molecule HLA-E. HLAE-self-peptide complexes can interact with CD94/NKG2 receptors expressed on NK and $CD8^+$ T cells to regulate their activities. Interactions between the killer inhibitory complex, CD94/NKG2A, result in the inhibition of $CD8^+$ T cell cytotoxicity against target cells, which in this case were myelin-reactive $CD4^+$ T cells from MS patients. Consistent with this, Correale and Villa found significantly elevated expression of CD94/NKG2A on $CD8^+$ T regs in patients during exacerbations of MS. Moreover, the cytolytic activity of the $CD8^+$ T regs from MS patients could be blocked with anti-HLA-E antibody, which further supports the role of HLA-E-restricted $CD8^+$ T regs in MS.

Glatiramer acetate (GA) is a synthetic copolymer that was discovered in studies on the treatment of EAE in rodents and shares key amino acid sequences with myelin basic protein. GA has received FDA approval as an immunotherapy for the treatment of MS, yet until recently, GA's mode of action has been unclear. However, Tennakoon and co-workers reported that injection of GA induced $CD8^+$ T regs that killed GA-reactive $CD4^+$ T cells from MS patients[28]**. GA-induced $CD8^+$ T regs expressed upregulation of perforin and killed GA-loaded $CD4^+$ T cells in a GA-specific manner. Moreover, like the study by Correale and Villa [27], this investigation demonstrated that the cytolysis mediated by $CD8^+$ T regs could be blocked with anti-HLA-E antibody. Remissions in MS in patients who were treated with GA were correlated with the spontaneous acquisition of GA peptide-induced $CD8^+$ T regs that were HLA-E-restricted and acted to eliminate $CD4^+$ pathogenic T cells[28,30].

$CD8^+$ T regs that Arise Spontaneously in Tumor-Bearing Hosts

A recent report from Jarnicki and co-workers uncovered an unusual compartmentalization of $CD8^+$ T reg activity in murine CT26 colon cancer[8]**. Injection of OVA into subcutaneous (SC) tumors resulted in the impaired OVA-specific T cell proliferation and reduced secretion of IFN-γ. CT26 tumor cells injected intravenously produced lung tumors that secreted TFG-β, which induced the production of IL-10 and TGF-β by tumor-infiltrating $CD4^+$ and $CD8^+$ T cells. In addition, resident APC in the lungs secreted high levels of IL-10, which further drove the *in situ* generation of IL-10-producing $CD8^+$ T regs[31]. Although both lung and SC tumors induced T regs, *in vivo* depletion of $CD8^+$ T cells resulted in a sharp decrease in lung tumors, but increased growth of SC tumors. Depletion of $CD8^+$ T regs resulted in enhanced secretion of IFN-γ by $CD4^+$ T cells in the lungs and a commensurate reduction in the growth of lung

tumors. By contrast, depletion of $CD8^+$ T cells in SC tumor-bearing mice led to a steep reduction in tumor-specific CTL and enhanced growth of the SC tumors. This study was the first to report $CD8^+$ T regs in the anti-tumor response in the lungs. Generation of $CD8^+$ T regs by lung tumors is reminiscent of the $CD8^+$ T regs induced in ACAID. The AC of the eye contains high levels of TGF-β which stimulates IL-10 production by resident APC and renders them tolerogenic. In CT26 lung tumors, resident APC already express elevated levels of IL-10 [31], which are driven even higher by TGF-β that is produced by the lung tumors. In both ACAID and pulmonary CT26 tumors, IL-10 secreting APC induce the generation of $CD8^+$ T regs. Thus, the local tissue environment can have a profound effect in shaping the immune response through the preferential induction of $CD8^+$ T regs.

Neither Fish nor Foul: Tumor-Promoting $CD8^+$ T Cells without Immunoregulatory Activity

The association between inflammation and cancer has been recognized for over a century and has led some to suggest that under some circumstances, a weak immune response promotes, rather than inhibits the development of cancer[32–34]. Recent studies demonstrated that αβ T cell deficiency was associated with reduced progression of chemically induced cancers[35, 36]. Two explanations come to mind to account for this curious finding: a) eliminating the αβ T cell population removes a population of T regs that restricts innate immune effector cells (e.g., NKT cells and γδ T cells) or b) a subpopulation of αβ T cells express tumor-promoting properties. Roberts and co-workers found that the growth of chemically induced tumors was stunted in $CD8^{-/-}$ mice compared to $CD4^{-/-}$ mice and wild-type syngeneic mice[36]**. Tumor growth was similarly reduced in $TCR\beta^{-/-}$ mice. However, $TCR\beta^{-/-}$ mice reconstituted with αβ TCR $CD8^+$ T cells exhibited increased tumor growth similar to wild-type mice. Analysis of tumor-infiltrating lymphocytes (TIL) in chemically induced tumors in wild-type mice revealed the presence of $CD8^+$ T cells that expressed a surface marker phenotype consistent with effector-memory cells ($CD44^+CD62L^-$). The $CD8^+$ TIL produced high amounts of IFN-γ and TNF-α mRNA when stimulated with anti-CD3. The $CD8^+$ TIL also produced COX-2, a major enzymatic inducer of inflammatory mediators and a molecule associated with tumor development. Interestingly, it has been reported that TNF-$\alpha^{-/-}$ mice[37] and $COX\text{-}2^{-/-}$ mice are resistant to chemically induced carcinomas[38]. Thus, the tumor-promoting activity of $CD8^+$ T cells might be mediated via TNF-α- and COX-2-dependent processes. These results are reminiscent of the "immune stimulation" of cancer hypothesis proposed by Prehn over 30 years ago and re-defined last year [33].

Conclusions

IFN-γ plays a critical role in either the induction or the expression of $CD8^+$ T reg activity. On first blush this seems counterintuitive considering the pro-inflammatory properties of IFN-γ. IFN-γ does not act as the end stage suppressive molecule, but functions indirectly by co-opting other cells to produce immunosuppressive and anti-inflammatory molecules such as TGF-B, IL-10, and IDO. Local production of these molecules creates *ad hoc* immune privileged sites. The challenge ahead is to design strategies that mimic $CD8^+$ T regs and to directly deliver these suppressor molecules in proper quantities and times to organs under autoimmune attack or organ allografts undergoing rejection.

Acknowledgements

Supported by NIH grants EY007641, EY005631, and EY016664 and an unrestricted grant from Research to Prevent Blindness.

References and Recommended Reading

1. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995;155:1151–1164. [PubMed: 7636184]
2. Menoret A, Myers LM, Lee SJ, Mittler RS, Rossi RJ, Vella AT. TGFbeta protein processing and activity through TCR triggering of primary CD8+ T regulatory cells. J Immunol 2006;177:6091–6097. [PubMed: 17056535]

**3. Myers L, Croft M, Kwon BS, Mittler RS, Vella AT. Peptide-specific CD8 T regulatory cells use IFN-gamma to elaborate TGF-beta-based suppression. J Immunol 2005;174:7625–7632. [PubMed: 15944263] This study showed that manipulation of a co-stimulatory molecule (4-1BB) induces $CD8^+$ T regs that produced and responded to IFN-γ. This shows that a normally pro-inflammatory cytokine, IFN-γ, produced by T regs induces an immunosuppressive molecule (TGF-β), which acts as the end stage inhibitor of $CD4^+$ T cell responses.

4. Sugita S, Ng TF, Lucas PJ, Gress RE, Streilein JW. B7+ iris pigment epithelium induce CD8+ T regulatory cells; both suppress CTLA-4+ T cells. J Immunol 2006;176:118–127. [PubMed: 16365402]

*5. Ashour HM, Niederkorn JY. Peripheral tolerance via the anterior chamber of the eye: role of B cells in MHC class I and II antigen presentation. J Immunol 2006;176:5950–5957. [PubMed: 16670303] This study demonstrates a unique form of antigen presentation by B cells in which class I-restricted and class II-restricted antigens are presented to $CD8^+$ and $CD4^+$ T cells respectively, resulting in the generation of $CD8^+$ T regs and $CD4^+$ T regs.

6. Ashour HM, Niederkorn JY. Expansion of B cells is necessary for the induction of T-cell tolerance elicited through the anterior chamber of the eye. Int Arch Allergy Immunol 2007;144:343–346. [PubMed: 17671393]
7. Alatrakchi N, Graham CS, van der Vliet HJ, Sherman KE, Exley MA, Koziel MJ. Hepatitis C virus (HCV)-specific CD8+ cells produce transforming growth factor beta that can suppress HCV-specific T-cell responses. J Virol 2007;81:5882–5892. [PubMed: 17376924]

**8. Jarnicki AG, Lysaght J, Todryk S, Mills KH. Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. J Immunol 2006;177:896–904. [PubMed: 16818744] This study reports an unusual compartmentalization in the expression of $CD8^+$ Treg activity. $CD8^+$ T regs prevent immune control of CD26 lung tumors but do not inhibit the rejection of subcutaneous CD26 tumors. Depletion of $CD8^+$ T cells results in the accelerated growth of subcutaneous tumors and the simultaneous restraint of lung tumors.

**9. Guillonneau C, Hill M, Hubert FX, Chiffoleau E, Herve C, Li XL, Heslan M, Usal C, Tesson L, Menoret S, et al. CD40Ig treatment results in allograft acceptance mediated by CD8CD45RC T cells, IFN-gamma, and indoleamine 2,3-dioxygenase. J Clin Invest 2007;117:1096–1106. [PubMed: 17404623] This study demonstrates for the first time the induction of infectious tolerance that is mediated by $CD8^+$T regs and sustains heart allograft survival through 4 generations of recipients. The authors also demonstrate that $CD8^+$ T regs produce IFN-γ, which does not act as the end stage suppressor molecule, but serves to co-opt vascular endothelial cells of the heart allograft to produce IDO and thereby create a local immune privileged milieu.

10. Niederkorn JY. See no evil, hear no evil, do no evil: The lessons of immune privilege. Nat Immunol. 2006
11. Niederkorn JY. Anterior chamber-associated immune deviation and its impact on corneal allografts. Curr Opin Organ Transplantation 2006;11:360–365.
12. Faunce DE, Sonoda KH, Stein-Streilein J. MIP-2 recruits NKT cells to the spleen during tolerance induction. J Immunol 2001;166:313–321. [PubMed: 11123307]
13. Wang Y, Goldschneider I, Foss D, Wu DY, O'Rourke J, Cone RE. Direct thymic involvement in anterior chamber-associated immune deviation: evidence for a nondeletional mechanism of centrally induced tolerance to extrathymic antigens in adult mice. J Immunol 1997;158:2150–2155. [PubMed: 9036960]

14. D'Orazio TJ, Niederkorn JY. Splenic B cells are required for tolerogenic antigen presentation in the induction of anterior chamber-associated immune deviation (ACAID). Immunology 1998;95:47–55. [PubMed: 9767456]

15. Nakamura T, Sonoda KH, Faunce DE, Gumperz J, Yamamura T, Miyake S, Stein-Streilein J. CD4+ NKT cells, but not conventional CD4+ T cells, are required to generate efferent CD8+ T regulatory cells following antigen inoculation in an immune-privileged site. J Immunol 2003;171:1266–1271. [PubMed: 12874214]

16. Skelsey ME, Mayhew E, Niederkorn JY. CD25+, interleukin-10-producing CD4+ T cells are required for suppressor cell production and immune privilege in the anterior chamber of the eye. Immunology 2003;110:18–29. [PubMed: 12941137]

17. Streilein JW, Niederkorn JY. Characterization of the suppressor cell(s) responsible for anterior chamber-associated immune deviation (ACAID) induced in BALB/c mice by P815 cells. J Immunol 1985;134:1381–1387. [PubMed: 3155766]

18. Skelsey ME, Mellon J, Niederkorn JY. Gamma delta T cells are needed for ocular immune privilege and corneal graft survival. J Immunol 2001;166:4327–4333. [PubMed: 11254685]

19. Xu Y, Kapp JA. gammadelta T cells are critical for the induction of anterior chamber-associated immune deviation. Immunology 2001;104:142–148. [PubMed: 11683953]

20. Skelsey ME, Mayhew E, Niederkorn JY. Splenic B cells act as antigen presenting cells for the induction of anterior chamber-associated immune deviation. Invest Ophthalmol Vis Sci 2003;44:5242–5251. [PubMed: 14638723]

21. Brandes M, Willimann K, Moser B. Professional antigen-presentation function by human gammadelta T Cells. Science 2005;309:264–268. [PubMed: 15933162]

22. Ashour HM, Niederkorn JY. Gammadelta T cells promote anterior chamber-associated immune deviation and immune privilege through their production of IL-10. J Immunol 2006;177:8331–8337. [PubMed: 17142729]

23. Faunce DE, Stein-Streilein J. NKT cell-derived RANTES recruits APCs and CD8+ T cells to the spleen during the generation of regulatory T cells in tolerance. J Immunol 2002;169:31–38. [PubMed: 12077225]

24. Sonoda KH, Faunce DE, Taniguchi M, Exley M, Balk S, Stein-Streilein J. NK T cell-derived IL-10 is essential for the differentiation of antigen- specific T regulatory cells in systemic tolerance. J Immunol 2001;166:42–50. [PubMed: 11123275]

**25. Sonoda KH, Nakamura T, Young HA, Hart D, Carmeliet P, Stein-Streilein J. NKT cell-derived urokinase-type plasminogen activator promotes peripheral tolerance associated with eye. J Immunol 2007;179:2215–2222. [PubMed: 17675481] This study demonstrates for the first time the induction of "infectious tolerance" that is mediated by $CD8^+$T regs and sustains heart allograft survival through 4 generations of recipients. The authors also demonstrate that $CD8^+$ T regs produce IFN-γ, which does not act as the end stage suppressor molecule, but serves to co-opt vascular endothelial cells of the heart allograft to produce IDO and thereby create a local immune privileged milieu.

*26. Cone RE, Li X, Sharafieh R, O'Rourke J, Vella AT. The suppression of delayed-type hypersensitivity by CD8+ regulatory T cells requires interferon-gamma. Immunology 2007;120:112–119. [PubMed: 17052246] The authors demonstrate that $CD8^+$ T regs induced by antigens introduced into the anterior chamber of the eye require IFN-γ to suppress DTH responses *in situ*. However, IFN-γ itself does not suppress DTH but "licenses" $CD8^+$ T regs to suppress the expression of DTH responses in previously sensitized hosts. The suppression is neither perforin-mediated cytolysis nor FasL-induced apoptosis.

*27. Correale J, Villa A. Isolation and characterization of CD8+ regulatory T cells in multiple sclerosis. Journal Neuroimmunology. 2008in press. The authors of this study reported the spontaneous appearance of cytolytic $CD8^+$ T regs in patients with MS. Reduced levels of cytolytic $CD8^+$ T regs correlated with relapses in MS. The cytolysis is directed against myelin-reactive $CD4^+$ T cells, the cell population implicated in the pathogenesis of MS. The $CD8^+$ T regs demonstrated exquisite specific and were restricted by the non-classical MHC class Ib molecule, HLA-E.

**28. Tennakoon DK, Mehta RS, Ortega SB, Bhoj V, Racke MK, Karandikar NJ. Therapeutic induction of regulatory, cytotoxic CD8+ T cells in multiple sclerosis. J Immunol 2006;176:7119–7129. [PubMed: 16709875] MS patients were immunized with an FDA-approved peptide, glatiramer acetate (GA). Patients responded by generating $CD8^+$ T regs that lysed GA-reactive $CD4^+$ T cells.

Reduced MS symptoms correlated with the presence of the $CD8^+$ T regs. Like the study by Correale et al., cytolysis by the $CD8^+$ T regs was restricted by HLA-E.

29. Lee YH, Ishida Y, Rifa'i M, Shi Z, Isobe K, Suzuki H. Essential role of CD8+CD122+ regulatory T cells in the recovery from experimental autoimmune encephalomyelitis. J Immunol 2008;180:825–832. [PubMed: 18178821]
30. Karandikar NJ, Crawford MP, Yan X, Ratts RB, Brenchley JM, Ambrozak DR, Lovett-Racke AE, Frohman EM, Stastny P, Douek DC, et al. Glatiramer acetate (Copaxone) therapy induces CD8(+) T cell responses in patients with multiple sclerosis. J Clin Invest 2002;109:641–649. [PubMed: 11877472]
31. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. Nat Immunol 2001;2:725–731. [PubMed: 11477409]
32. Prehn RT. Perspectives on oncogenesis: does immunity stimulate or inhibit neoplasia? J Reticuloendothel Soc 1971;10:1–16. [PubMed: 4330591]
33. Prehn RT. Does the immune reaction cause malignant transformation by disrupting cell-to-cell or cell-to-matrix communications? Theor Biol Med Model 2007;4:16. [PubMed: 17480231]
34. Prehn RT, Lappe MA. An immunostimulation theory of tumor development. Transplant Rev 1971;7:26–54. [PubMed: 4947774]
35. Girardi M, Glusac E, Filler RB, Roberts SJ, Propperova I, Lewis J, Tigelaar RE, Hayday AC. The distinct contributions of murine T cell receptor (TCR)gammadelta+ and TCRalphabeta+ T cells to different stages of chemically induced skin cancer. J Exp Med 2003;198:747–755. [PubMed: 12953094]

**36. Roberts SJ, Ng BY, Filler RB, Lewis J, Glusac EJ, Hayday AC, Tigelaar RE, Girardi M. Characterizing tumor-promoting T cells in chemically induced cutaneous carcinogenesis. Proc Natl Acad Sci U S A 2007;104:6770–6775. [PubMed: 17412837] This paper reports a curious tumor promoting $CD8^+$ T cells that do not function as T regs *per se*, but promote tumor growth without affecting other T cells. That is, adoptive transfer of the $CD8^+$ T cells to αβ TCR $^{-/-}$ mice results in enhanced tumor growth and thus, demonstrates that these cells are not T regs, but are tumor promoters. The tumor-promoting activity of these $CD8^+$ T cells is reminiscent of the "immune stimulation" hypothesis for tumorigenesis that was articulated over 30 years ago.

37. Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M, et al. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med 1999;5:828–831. [PubMed: 10395330]
38. Tiano HF, Loftin CD, Akunda J, Lee CA, Spalding J, Sessoms A, Dunson DB, Rogan EG, Morham SG, Smart RC, et al. Deficiency of either cyclooxygenase (COX)-1 or COX-2 alters epidermal differentiation and reduces mouse skin tumorigenesis. Cancer Res 2002;62:3395–3401. [PubMed: 12067981]