

Molecular mechanisms in allergy and clinical immunology

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The role of suppressor T cells in regulation of immune responses

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Suppressor T cells play important roles in the regulation of immune responses and the mediation of dominant immunologic tolerance. Studies of suppressor T-cell function have been hampered until their recent identification as a minor fraction (approximately 10%) of CD4⁺ T cells that coexpress CD25. CD4⁺CD25⁺ T cells have been shown to play a critical role in the prevention of organ-specific autoimmunity and allograft rejection. Because tumor antigens are self-antigens, it is not surprising that CD4⁺CD25⁺ T cells also inhibit the induction of tumor immunity. The spectrum of activity of CD4⁺CD25⁺ cells extends to non-self-antigens, including infectious agents. Indeed, T cell-mediated suppression might be responsible for the low level of chronic infection seen with many pathogens. Interestingly, however, this persistent level of infection might be beneficial to the host and needed for maintenance of immunologic memory. Although CD4⁺CD25⁺ T cells are capable of inhibiting T_H2 responses, their role in the suppression of allergic responses has not been firmly established. Depending on the desired immune response, enhancement or restraint of suppressor T-cell function might be required. Therefore immunologic or pharmacologic manipulation of regulatory T-cell populations represents an important future approach to immunotherapy of a wide range of immune responses. (*J Allergy Clin Immunol* 2002;110:693-702.)

Key words: CD4⁺CD25⁺ T cells, immunoregulation, tolerance, autoimmunity, tumor immunity, chronic infection, allergy, immunotherapy

An important and still unsolved problem for immunologists and clinicians is natural tolerance to self-antigens. Models of passive-recessive tolerance, such as thymic deletion of autoreactive T cells or nonresponsiveness in the periphery because of anergy or ignorance, cannot account for the presence of circulating autoreactive cells in a host, leading to a small but significant incidence of autoimmunity in the population. The concept of the sup-

Abbreviations used

CTLA-4: Cytotoxic T-lymphocyte antigen 4
d3Tx: Thymectomy on the third day of life
GITR: Glucocorticoid-induced TNF receptor
GITR-L: Ligand for glucocorticoid-induced TNF receptor
GVHD: Graft-versus-host disease
HC: Hematopoietic cell
IPD: Inflammatory pulmonary reaction
MLR: Mixed lymphocyte culture reaction
RAG: Recombination activating gene
TCR: T-cell receptor
TGF: Transforming growth factor

pressor T cell has endured an arduous and controversial journey toward acceptance into mainstream immunology. The phenomenon of T-cell suppression of autoimmune disease was first described in the late 1960s. Nishizuka and Sakakura¹ found that thymectomy of mice on the third day of life (d3Tx) would result in organ-specific autoimmunity, but disease would not develop if the mice were thymectomized as early as day 2 or as late as day 7 of life. Suppression of d3Tx-induced autoimmunity could be achieved if the animal with d3Tx was reconstituted by 2 weeks of life with adult thymocytes or splenocytes from an adult animal but not with cells from an adult animal that had d3Tx.² These studies indicated qualitative differences between the cells emigrating from the thymus before and after the third day of life.

It was not until the mid-1990s that serious consideration was given to the thought of a suppressor T-cell population. Sakaguchi et al³ identified a subpopulation (approximately 10%) of CD4⁺ cells that expressed the IL-2 receptor α chain (CD25). Depletion of CD25⁺ cells from adult splenocytes and transfer of the resulting CD25⁻ T cells to immunocompromised hosts induced the same spectrum of autoimmune diseases as seen in animals with d3Tx. Cotransfer of CD4⁺CD25⁺ cells would inhibit the induction of autoimmunity in this cell-transfer model, as well as in the d3Tx model.⁴

The availability of a marker to define a subpopulation of T cells has certainly furthered their characterization as a distinct lineage of cells. Over the past decade, these cells have been functionally analyzed *in vitro*⁵⁻⁸ and described for their suppressive function in many experimental and spontaneous autoimmune diseases.^{3,4,9-12}

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TABLE I. In vitro and in vivo characteristics of CD4⁺CD25⁺ T cells

In vitro	In vivo
Considered anergic, no proliferation or IL-2 production in response to TCR stimulus	Absent or reduced numbers in animals deficient in IL-2, IL-2 receptors, costimulatory pathways (CD28, B71/2, CD40/CD154)
Inhibit IL-2 production of responding CD4 and CD8 T cells	Suppress autoreactive cells in d3Tx-induced organ-specific autoimmunity, IBD, IDDM
Induction of CD25 expression on CD25 ⁻ T cells does not enable suppressive phenotype	Generation in thymus by selection on self-peptide presented by MHC class II-positive cortical epithelial cells
IL-2, anti-CD28, anti-CTLA-4, anti-TGF- β , anti-GITR have overcome suppression	Anti-CTLA-4, anti-TGF- β , anti-IL-10 reverse suppression of IBD
TCR activation is required for suppressive activity	Evidence for recognition of tissue-specific antigens for autoimmune suppression
Mechanism of suppression is considered cell-contact dependent	Mechanism of suppression is undetermined, but antibodies to some cytokines can overcome suppression of autoimmune disease

IBD, Inflammatory bowel disease; IDDM, insulin-dependent diabetes mellitus.

Functionally and phenotypically similar cells have also been described in human subjects.¹³ CD4⁺CD25⁺ T cells are considered anergic because they do not proliferate or produce IL-2 in response to T-cell receptor (TCR) stimulus. They do, on TCR stimulus, suppress proliferation of responding CD4⁺ and CD8⁺ T cells by inhibiting the production of IL-2, as well as inhibiting the upregulation of IL-2 receptors. In vitro analysis reveals that their inhibition is through cell-cell contact and is not dependent on immunosuppressive cytokines. Their activity in vivo, however, seems to have a certain dependency on the production of IL-4, IL-10, and transforming growth factor (TGF) β .^{9,14,15}

In addition to the naturally suppressive CD4⁺CD25⁺ CD45RB^{lo} populations described above, other types of induced regulatory T cells have been described, including those generated by activation in the presence of IL-10 (T_R1 cells),¹⁶ oral tolerance,¹⁷ or repeated stimulations with immature dendritic cells.¹⁸ The establishment of an active mechanism for control of immune responses (ie, dominant tolerance) allows for an ideal target of therapeutic manipulation to enhance or reduce immune control.

Although much knowledge has been gained over the last decade of study (Table I), there are still many questions unanswered regarding the role of suppressor T cells. These have been recently addressed,¹⁹ but let us reiterate some of them here.

- How are suppressor T cells educated, generated, and maintained?
- What markers can be used to identify the suppressive subpopulations?
- How do they exert their suppressive functions?
- What is their antigen specificity?
- How do they distinguish a protective and productive immune response from a deleterious one?
- Can the regulators be regulated?

Recently, the potential for CD4⁺CD25⁺ T cell-mediated immunoregulation has extended beyond autoimmunity and into a broad scope of immune responses, includ-

ing tumor immunity and transplantation tolerance (Fig 1). This review will focus on the role CD4⁺CD25⁺ T cells, as well as other T-regulatory cells, play in these immune responses. It is also a goal to see whether the study of these cells in these immune models might help answer these questions. In addition, the manipulation of their function for therapeutic purposes in the vaccination against tumor antigens and the clearance or control of persistent bacterial infections will be addressed.

DEPLETION OF CD4⁺CD25⁺ REGULATORY T CELLS FOR TUMOR IMMUNITY

There is evidence that the immune response raised against tumor-expressed antigens can cross-react with normal tissue, resulting in tumor regression but also inducing autoimmunity.²⁰⁻²³ These findings indicate that tumor immunity and autoimmunity might be linked, and the control mechanisms leading to suppression of autoimmune effectors might also impede generation of an effector antitumor response. One might wonder, however, why the immune system would develop a mode of action that would protect against autoimmunity but leave a risk for tumor development. Perhaps this could be answered by analyzing when the 2 immune disorders occur in an individual. Many autoimmune diseases present on or before childbearing age, whereas tumors are considered a degenerative disease, occurring late in life. Therefore providing immune protection at the earlier stages in life would allow for childbirth and rearing.

Regulatory T cells can potentially inhibit immune responses to many self-antigens. Recently, it has been described that CD4⁺CD25⁺ T cells also suppress immune responses to tumor antigens. The evidence for their action in controlling activation of tumor-specific effector cells comes from models of CD25⁺ cell depletions (Fig 2).²⁴⁻²⁷ Suppression of an aggressive antitumor response was actually described over 20 years ago.²⁸ North et al^{29,30} described the induction of a population of CD4⁺ T

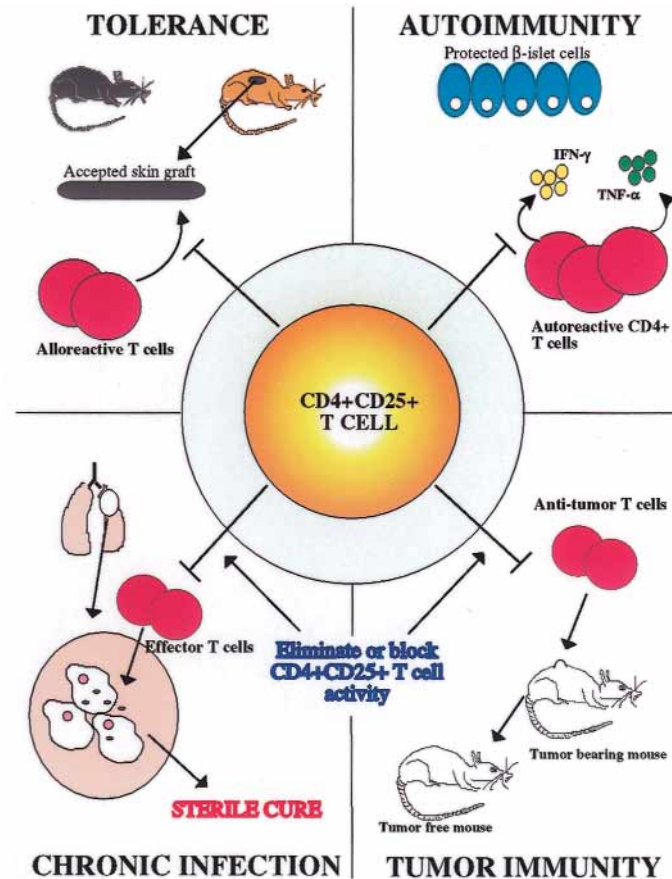


FIG 1. Summary of immune responses that have demonstrated CD4+CD25+ T cell-mediated suppression. CD4+CD25+ T cells have suppressive functions in many immune responses. These cells have beneficial roles, including prevention of autoimmune disease and promotion of graft acceptance. These cells, however, also suppress some beneficial immune responses, such as those to tumor and bacterial antigens. By eliminating or inactivating their suppressive function, it is possible to enhance effector immune responses to these antigens.

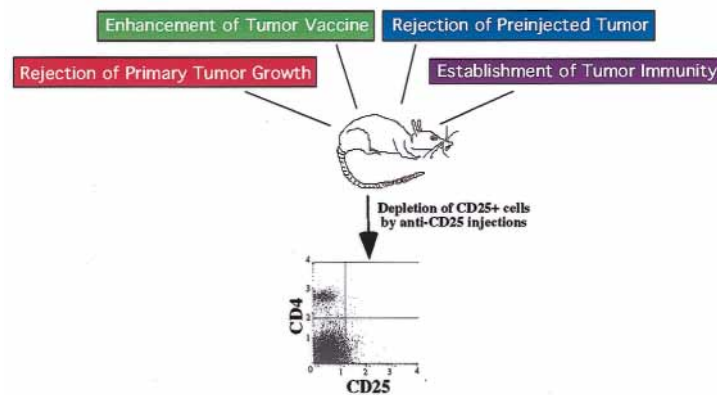


FIG 2. Depletion of CD4+CD25+ T cells enhances immunity to tumors. Adult mice are depleted of CD25+ cells through repeated anti-CD25 antibody injections. Depleted animals are challenged with tumor cells or tumor vaccines, followed by tumor challenge. Mice efficiently depleted of CD25+ cells have enhanced responses against primary and established tumors.

cells from tumor-bearing mice that can downregulate the effector immune response against Meth-A sarcoma. This tumor-induced suppression could be broken by using similar methods to break tolerance for the induction of autoimmunity. Treatment with cyclophosphamide or irradiation could break the action of these immunosuppressive T cells and allow regression of various tumors.^{31,32}

The majority of the recent studies to improve responses to tumor antigens and tumor-based vaccines have focused on selective depletion of CD25⁺ cells. Taguchi and Takahashi³³ previously demonstrated that in vivo elimination of CD25⁺ cells by means of antibody injection could spontaneously lead to organ-specific autoimmune disease, indicating that the mere depletion of CD25⁺ cells from the CD4⁺ T-cell repertoire could break tolerance to self-antigens, inducing an effector immune response. Many investigators took advantage of this phenomena and eliminated regulatory cells, resulting in protection from several types of tumors.²⁴⁻²⁶ Surprisingly, the selective depletion of CD25⁺ cells that could induce tumor immunity did not induce organ-specific autoimmune disease in these studies.

In addition to preventing the growth of primary tumors, depletion of CD25⁺ T cells enhanced the immunogenicity of tumor vaccines, as well as treatment of recently established tumors. B16 tumors expressing either IFN- α or GM-CSF were prevented from growing in CD25⁺ cell-depleted animals. In addition, injection of anti-CD25 antibody in combination with repeated injection of a B16 tumor expressing GM-CSF improved regression of a preinjected B16 tumor.²⁷ Survival of mice was even further improved with the combination of CD25⁺ cell depletion and cytotoxic T-lymphocyte antigen 4 (CTLA-4) blockage.

The potential control of human tumors by CD4⁺CD25⁺ cells has recently been described. Woo et al³⁴ were able to isolate CD4⁺CD25⁺ cells that coexpress CTLA-4 from lung tumor tissue. These cells were shown to have suppressive effects in vitro on autologous, but not allogenic, T cells, indicating a level of antigen specificity in the enriched CD4⁺CD25⁺ T-cell population. Although there is evidence of tissue specificity in autoimmune models,^{35,36} the antigen specificity of tumor-suppressive CD4⁺CD25⁺ T cells has yet to be determined. Would injection of tumor cells generate tumor antigen-specific suppressors, or are CD4⁺CD25⁺ T cells triggered by activation of tumor-self-specific T cells? In vitro functional analysis⁷ has shown that once activated CD4⁺CD25⁺ T cells are activated through their TCR, their ability to suppress an immune response is antigen independent. Does this property also occur in vivo? These questions still remain to be addressed.

With the potential for CD25⁺ T cells to regulate responses to tumors in human subjects, one could envision tumor immunotherapy or vaccination in combination with removal or inactivation of CD25⁺ T cells. It has been confirmed that CD25⁺ cell depletion from host animals will not, by itself, induce autoimmune disease. In combination with strong TCR stimuli, however, absence of CD4⁺CD25⁺ regulatory cells can result in multiorgan specific autoim-

mune disease.³⁷ Therefore inactivation or removal of regulatory cells as a therapeutic enhancement for an antitumor immune response should proceed with caution.

REGULATORY T CELLS CONTROLLING TRANSPLANT TOLERANCE

The idea of an active mechanism for transplant acceptance has also been theorized for many years.^{38,39} Manipulations, such as depleting CD4 antibody injections or blockade of CD28/B7 or CD40/CD40L interactions in combination with transfer of donor hematopoietic cells (HCs), have promoted allograft survival in many species.⁴⁰ Splenocytes from these long-term survivors confer transplant tolerance to syngeneic recipients. These cells that transfer active tolerance were described as CD4⁺ T cells, but analysis in vitro revealed that these T cells could proliferate and produce inflammatory cytokines in response to the alloantigens.⁴¹ The majority of these early studies analyzed unseparated T-cell populations, and therefore the distinction between alloreactive memory cells, naive autoreactive T cells, and transplant-induced regulatory T cells was uninterpretable.

Markers that delineate these cells were used to subdivide T cells from tolerant animals into CD4⁺CD45RB^{hi}, CD4⁺CD45RB^{lo}, and CD4⁺CD25⁺ subsets to investigate the role regulatory T cells play in transplant tolerance. Hara et al,⁴² using a protocol of depleting anti-CD4 mAb in combination with HC transfer for cardiac allograft survival, were able to determine that the CD45RB^{hi} and CD45RB^{lo} subsets from tolerant donors responded differently in vitro to allogeneic stimulation. Whereas CD4⁺CD45RB^{hi} T cells from both tolerant and naive mice produced IL-2 and IFN- γ in response to the alloantigen, the CD4⁺CD45RB^{lo} subpopulation failed to proliferate or produce either T_H1 or T_H2 cytokines. CD4⁺CD45RB^{lo} and CD4⁺CD25⁺ but not CD4⁺CD25⁻ T cells from tolerant donors were found to confer tolerance to syngeneic recipients through an IL-10-dependent mechanism. CD4⁺CD25⁺ T cell-mediated transplant tolerance is dependent on both IL-10 and CTLA-4 because blockade of either pathway completely abrogated transplant survival.⁴³ In a model using donor cell infusion with anti-CD154 treatment,⁴⁴ anti-CTLA-4 treatment only had effects on inhibiting tolerance when used at the induction phase. Once tolerance was established, no effect of anti-CTLA-4 or anti-CD25 treatment was observed.

Kingsley et al⁴³ further defined that CD4⁺CD25⁺ T cells were generated using the tolerance induction protocol before allograft transplantation. This exposure was critical because enriched CD4⁺CD25⁺ cells from naive animals could not suppress allograft rejection, indicating the necessity of stimulation with specific antigens. Additionally, tolerized animals did not exert universal immunosuppression because third-party grafts could be readily rejected. Therefore the regulatory cells seem to be H-2 specific, but are they also tissue specific? Recently, regulatory cells have been suggested to reside and be maintained within the tolerized graft itself⁴⁵ and can transfer tolerance to a naive recipient. This suggests that

retention within the tissue is possibly mediated by TCR interactions with MHC. Although initial tolerizing tissue exposure involved HCs, it is unknown whether the regulatory T cells are only specific for donor antigens expressed on HCs or also acquire specificity for non-HC tissue-specific antigens.

Taylor et al⁴⁶ investigated how CD4⁺CD25⁺ T cells played a role in tolerance induced in vitro by establishing a mixed lymphocyte culture reaction (MLR) in the presence of antibodies blocking CD28/B7 or CD40/CD154 costimulatory pathways. Alloreactive CD4⁺ T cells stimulated under these conditions would not induce graft-versus-host disease (GVHD) on transfer to recipient animals. It was crucial, however, that CD4⁺CD25⁺ T cells were present in this in vitro culture. Depletion of CD4⁺CD25⁺ T cells before the MLR and costimulatory blockage induced CD4⁺ T cells that could mediate GVHD. Mechanistically, this tolerance-inducing regimen is still undefined. Do the blocking antibodies have a direct effect on enhancing CD4⁺CD25⁺ T-cell function, or are these cells necessary to downregulate IL-2 production in alloreactive CD4⁺ T cells that, in combination with costimulatory blockade, induce tolerance? It still remains to be determined whether naive CD4⁺CD25⁺ T-cell reconstitution to animals receiving the GVHD-inducing cells would have an effect on host survival. In addition, it is unclear whether removal of CD4⁺CD25⁺ T cells after the tolerance-inducing MLR would have any effect on GVHD in the recipient animal.

Although the previous studies found no role for CD4⁺CD25⁺ cells in transplant tolerance, a recent study⁴⁷ on skin transplantation found that both CD4⁺CD25⁺ and CD4⁺CD25⁺ cells could regulate transplant rejection, although the CD25⁺ subpopulation required 10-fold greater numbers for suppression. This model of CD25⁺ T cell-mediated transplant tolerance, unlike the models described earlier, has no dependence on IL-10 or CTLA-4. Indeed, the study by Graca et al⁴⁷ investigated tolerance to only minor H-2 differences, whereas Kingsley et al⁴³ used full H-2 differences. Therefore it is possible that differences in the tolerance induction protocol or the origin of the allograft have alternate requirements for suppression. This is true for different autoimmune diseases because inflammatory bowel disease is dependent on IL-10 and TGF- β expression, but autoimmune gastritis is not.^{48,49}

The regulatory populations defined by Graca et al⁴⁷ represent 2 distinct populations with likely different modes of action. Serial analysis of gene expression revealed that the CD25⁺ and CD25⁺ subpopulations have different patterns of gene expression. A more detailed serial analysis of gene expression comparison of various populations of regulatory T cells and freshly isolated CD4⁺CD25⁺ and CD4⁺CD25⁺ cell populations, as well as rejecting syngeneic and tolerized skin grafts, was performed.⁵⁰ Many genes found to be upregulated in the tolerized skin are also present in the naturally suppressive CD4⁺CD25⁺ population,^{51,52} including preproenkephalin, CD103, and a member of the TNF super-

family, glucocorticoid-induced TNF receptor (GITR). However, many of these genes have also been identified in activated CD4⁺CD25⁺ T-cell populations,⁵¹ and therefore it is difficult to determine whether the regulatory cells present in tolerized skin are derived from the naturally suppressive CD4⁺CD25⁺ population or arose through activation of another population of CD4⁺ T cells.

Some analyses of CD4⁺CD25⁺ T cells in human subjects have revealed that they have the capacity to recruit CD4⁺CD25⁺ T cells to become suppressive.^{53,54} These CD25⁺ suppressor cells seem to mediate their action through soluble inhibitory cytokines, such as IL-10 or TGF- β . Could a similar phenomenon be taking place within transplant models in the mouse? Many of these models are highly dependent on IL-10 production, and various means of inducing IL-10 production within the regulatory subpopulations seem to enhance tissue engraftment.^{55,56}

REGULATION OF IMMUNE RESPONSES TO INFECTIOUS DISEASE

All the above-described immune responses controlled by CD4⁺CD25⁺ regulatory T cells have been against self-antigens, either by suppressing autoimmune disease or immune responses to tumors or by enhancing graft acceptance. Immunologists and clinicians have many questions concerning other roles these cells might play in immune responses.

- Can their ability to control immune responses go beyond suppression of self-reactivity into the realm of an effector response against foreign antigens and pathogens? In initial analysis of CD4⁺CD45RB^{lo} regulatory cells, it was demonstrated in the susceptible BALB/c model of *Leishmania major* infection that there was a population of *L major*-specific T cells that could suppress the T_H1 effector T cells.⁵⁷ In addition, inflammatory bowel disease is dependent on microflora located in the gut,⁵⁸ indicating that suppression could result in control of both autoreactive and non-self-specific T cells that induce wasting disease.
- Can clinicians exploit the properties of CD4⁺CD25⁺ regulatory T cells to control immune responses that result in tissue pathology? Indeed, it has been theorized⁵⁹ that the presence of regulatory T cells is not to exclusively control responses to self-antigens but to adjust all immune responses to an appropriate level.
- Additionally, do bacterial or viral pathogens use regulatory T cells to promote their growth within the host?

Many laboratories are beginning to investigate these questions. Recently, it has been demonstrated that CD4⁺CD25⁺ T cells can prevent the CD4 T-cell mediated immunopathology and death after *Pneumocystis carinii* infection.⁶⁰ This microorganism is nonpathogenic in immunocompetent hosts; however, if infected recombination activating gene (RAG)^{-/-} hosts are reconstituted with naive CD4⁺ T cells, a massive inflammatory pulmonary reaction (IPD) will kill the animal within a few weeks.⁶¹ Reconstitution of infected RAG^{-/-} animals

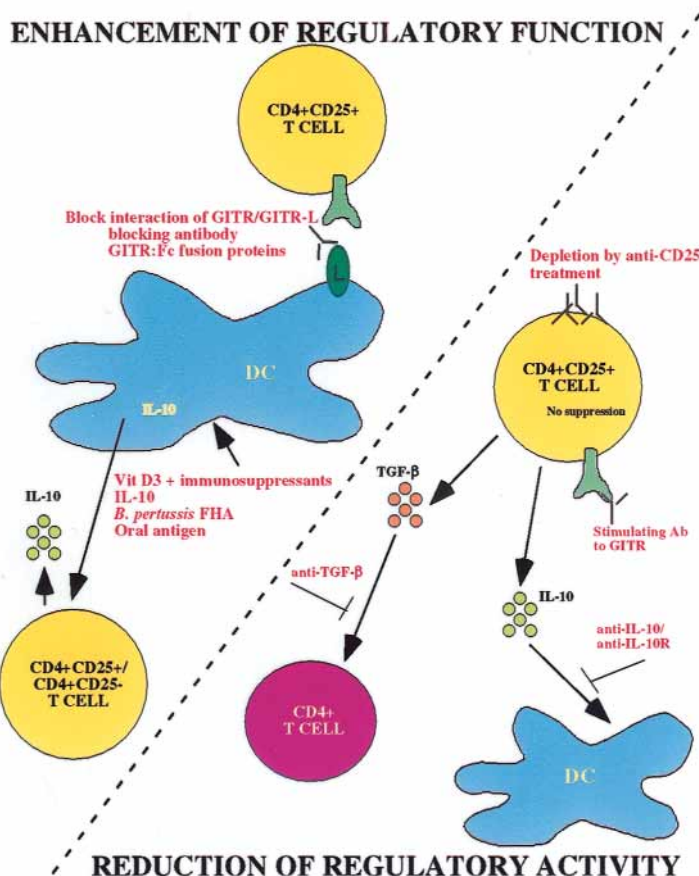


FIG 3. Possible manipulation of regulatory T-cell activity to enhance or reduce immune responses. Reduction of CD4+CD25+ T-cell activity by means of cell depletion, GITR signaling, or blocking effector cytokine function might serve as a target to enhance immune responses to tumors or vaccination. Enhancement of regulatory T-cell function by means of blocking GITR signaling or modifying antigen-presenting cells to generate IL-10-producing regulatory cells might serve as a target for downregulation of autoimmune or alloreactive effector cells, as well as control the inflammatory response to foreign antigen.

with CD4+CD25+ T cells does not result in IPD and can actually control IPD induced by naive CD4+CD25- T cells.⁶⁰ In addition to controlling the acute lethal pathology caused by the immune response of the CD4 T cells, clearance of the microorganism is also prevented, and therefore CD4+CD25+ T cells seem to play a role in the maintenance of chronic infections.

Indeed, maintenance of a low, persistent level of infection is seen with many pathogens, including *Mycobacterium tuberculosis*,⁶² HIV,⁶³ and *L major*.⁶⁴ In the *L major*-resistant mouse strain C57BL/6, there is an initial increase in parasite load after infection. This induces a strong T_H1 immune response that does result in a healing lesion, but a low level of parasite persists within this lesion, which is dependent on IL-10 production.⁶⁵ By using a cell-transfer model, reconstitution of *L major*-infected RAG^{-/-} animals with CD4+CD25- T cells completely clears the infection. Cotransfer of CD4+CD25+ T cells results in a healing lesion, with persistent low par-

asite load in the host. The suppression of a sterilizing immunity is dependent on IL-10 as a treatment with anti-IL-10, or the use of IL-10^{-/-} CD4+CD25+ T cells results in complete clearance of the parasite from the host.⁶⁶

Why would CD4+CD25+ T cells play a role in allowing a protective immune response to invading pathogens to occur but suppress complete clearance of the antigen? Interestingly, in the *L major* model the persistent level of parasite is required for induction of long-term immunity. If, in the cell-transfer model, the parasite load is completely cleared by CD4+ T cells, a memory response to the pathogen is not generated or maintained. Challenge of healed IL-10^{-/-} animals or healed wild-type animals treated with anti-IL-10 receptor resulted in a response similar to that after a primary infection. There was no evidence of an *L major* memory response. The necessity to allow immune protection from the invading pathogen and immune control for sustained antigen presence indicates a delicate regulatory balance, the maintenance of which is presently unknown.

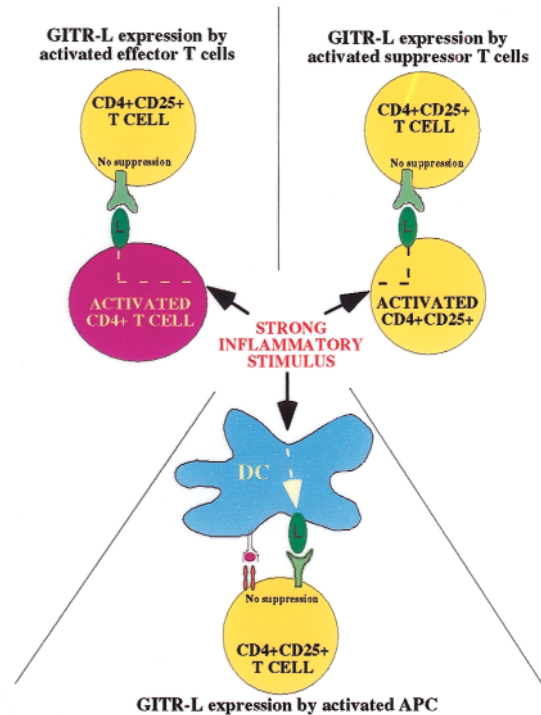


FIG 4. Models of GITR-L expression and interaction with GITR on CD4+CD25+ T cells. For in vivo control of CD4+CD25+ T-cell activity, GITR-L might be induced on various cell types to turn off suppressive activity. Perhaps a strong inflammatory stimulus might upregulate GITR-L on effector T cells or antigen-presenting cells to instruct CD4+CD25+ T cells to turn off their regulatory function. In turn, GITR-L might be upregulated on activated suppressor cells to serve as a negative feedback loop for suppressive activity.

Unfortunately, the regulatory T cell's ability to suppress an immune response might be exploited by pathogens to enhance their growth within a host. Recent studies on chronic Friend retroviral infection⁶⁷ and hepatitis C virus infection⁶⁸ demonstrate that regulatory T cells can be generated and play a role in suppression of the antiviral immune response. In addition to virus-induced regulatory T cells, *Bordetella pertussis* might promote the generation of pathogen-specific T_R1 cells by modifying dendritic cells in the lung to secrete IL-10.⁶⁹ Therefore depending on the pathogen in question, it might be necessary to reduce or enhance the activity of regulatory T cells to achieve an appropriate immune response.

DO REGULATORY T CELLS PLAY A ROLE IN ALLERGIC RESPONSES?

One of the most clinically relevant effector responses is immune hypersensitivity. In developed nations the incidence of allergy, especially asthma, has been on the rise for several decades. The responses to allergens are typically of the T_H2 phenotype,⁷⁰ with IL-4, IL-5, and IL-13 playing roles in IgE isotype switching and eosinophil recruitment to the site of inflammation.⁷¹⁻⁷⁴

TABLE II. Therapeutic implications of CD4+CD25+ T cells

Reduction of CD4+CD25+ regulatory function
• Enhancement of tumor immunity
• Clearance of persistent infection—sterilizing immunity
• Improvement of responses to vaccines
Enhancement of CD4+CD25+ regulatory function
• Treatment of autoimmunity
• Treatment of allergic responses
• Inducement of transplant tolerance
• Control of pathogenic inflammatory responses to foreign antigen

CD4+CD25+ T cells have been shown to regulate T_H2 immune responses⁷⁵; however, their role in control of allergic responses has yet to be defined.

Cottrez et al⁷⁶ has demonstrated that regulatory T cells can modify allergic responses in mice. By using an ovalbumin-specific T_R1 clone developed to exclusively produce IL-10 on antigen challenge, this group was able to downregulate antigen-specific IgE responses induced by immunization of ovalbumin in alum. These regulatory cells, however, were derived in vitro, making the use of this type of clone for human therapeutics unlikely.

Recently, Suto et al⁷⁷ looked at a role of CD4⁺CD25⁺ T cells in an airway hypersensitivity model. By using an *in vivo* CD25⁺ cell-depletion protocol and cell transfer, it was demonstrated that antigen-induced eosinophil recruitment was enhanced in the absence of CD25⁺ T cells. In these studies, however, direct suppression of airway hypersensitivity by CD25⁺ T cells was not demonstrated.

CONCLUSIONS

Evidence presented in this review establishes a role for CD4⁺CD25⁺ T cells in multiple effector responses beyond autoimmunity. Can they be used as a target for therapeutic manipulation of immune responses? Depending on the desired immune response, it would be necessary to enhance or suppress the function of the regulatory T cells (Fig 3). Indeed, removal of these cells has been shown to enhance immune responses to both tumor and foreign antigens. In addition to depletion, could their function be downregulated by other means? Recently, 2 groups independently demonstrated that engagement and signaling through GITR on the surface of CD4⁺CD25⁺ T cells could temporarily turn off their suppressive function *in vitro*.^{51,78} It still remains to be determined how GITR interactions with its ligand (GITR-L) *in vivo* might downregulate CD4⁺CD25⁺ functions. Some models describing where GITR-L might be expressed and how it might interact with CD4⁺CD25⁺ T cells are shown in Fig 4. In turn, blocking the natural interaction between GITR and GITR-L might enhance their suppressive abilities. Additionally, other methods, such as treatment with an active form of vitamin D3 and the immunosuppressant mycophenolate mofetil,⁵⁵ might enhance regulatory T-cell function by manipulating local dendritic cells to secrete IL-10 without IL-12. Clearly, now that CD4⁺CD25⁺ T cells have been accepted as a unique lineage of suppressor T cells, investigation should be considered on how manipulating their function can be used in the clinical setting (Table II).

REFERENCES

- Nishizuka Y, Sakakura T. Thymus and reproduction: sex-linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science* 1969;166:753-5.
- Kojima A, Tanaka-Kojima Y, Sakakura T, Nishizuka Y. Prevention of post-thymectomy autoimmune thyroiditis in mice. *Lab Invest* 1976;34:601-5.
- Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunological 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;155:1151-64.
- Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. CD4⁺CD25⁺ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J Immunol* 1998;160:1212-8.
- Thornton AM, Shevach EM. CD4⁺CD25⁺ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 1998;188:287-96.
- Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, et al. Immunologic self-tolerance maintained by CD25⁺CD4⁺ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998;10:1969-80.
- Thornton AM, Shevach EM. Suppressor effector function of CD4⁺CD25⁺ immunoregulatory T cells is antigen nonspecific. *J Immunol* 2000;164:183-90.
- Piccirillo C, Shevach E. Cutting edge: control of CD8⁺ T cell activation by CD4⁺CD25⁺ immunoregulatory cells. *J Immunol* 2001;167:1137-40.
- Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med* 1999;190:995-1003.
- Read S, Malmstrom V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25⁺CD4⁺ regulatory cells that control intestinal inflammation. *J Exp Med* 2000;192:295-302.
- Stephens LA, Mason D. CD25 is a marker for CD4⁺ thymocytes that prevent autoimmune diabetes in rats, but peripheral T cells with this function are found in both CD25⁺ and CD25⁻ subpopulations. *J Immunol* 2000;165:3105-10.
- Salomon B, Lenschow DJ, Rhee L, Ashourian N, Singh B, Sharpe A, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4⁺CD25⁺ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000;12:431-40.
- Shevach EM. Certified professionals: CD4⁺CD25⁺ suppressor T cells. *J Exp Med* 2001;193:F41-5.
- Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL. A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4⁺ T cells. *J Exp Med* 1996;183:2669-74.
- Seddon B, Mason D. Regulatory T cells in the control of autoimmunity: the essential role of transforming growth factor beta and interleukin 4 in the prevention of autoimmune thyroiditis in rats by peripheral CD4⁺CD45RC⁻ cells and CD4⁺CD8⁻ thymocytes. *J Exp Med* 1999;189:279-88.
- Groux H, Ogara A, Bigler M, Rouleau M, Antonenko S, deVries JE, et al. A CD4⁺ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997;389:737-42.
- Zhang X, Izikson L, Lui L, Weiner HL. Activation of CD25⁺CD4⁺ regulatory T cells by oral antigen administration. *J Immunol* 2001;167:4245-53.
- Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10-producing, nonproliferating CD4⁺ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J Exp Med* 2000;192:1213-22.
- Shevach EM. CD4⁺CD25⁺ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2:389-400.
- Hara I, Takechi Y, Houghton AN. Implicating a role for immune recognition of self in tumor rejection—passive-immunization against the brown locus protein. *J Exp Med* 1995;182:1609-14.
- Geissler M, Mohr L, Weth R, Kohler G, Grimm CF, Krohne TU, et al. Immunotherapy directed against alpha-fetoprotein results in autoimmune liver disease during liver regeneration in mice. *Gastroenterology* 2001;121:931-9.
- Yee C, Thompson JA, Roche P, Byrd DR, Lee PP, Piepkorn M, et al. Melanocyte destruction after antigen-specific immunotherapy of melanoma: direct evidence of T cell-mediated vitiligo. *J Exp Med* 2000;192:1637-43.
- Ludewig B, Ochsenbein AF, Odermatt B, Paulin D, Hengartner H, Zinkernagel RM. Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. *J Exp Med* 2000;191:795-803.
- Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T, Nakayama E. Tumor rejection by *in vivo* administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. *Cancer Res* 1999;59:3128-33.
- Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25⁺CD4⁺ T cells: a common basis between tumor immunity and autoimmunity. *J Immunol* 1999;163:5211-8.
- Steitz J, Bruck J, Lenz J, Knop J, Tuting T. Depletion of CD25⁺CD4⁺ T cells and treatment with tyrosinase-related protein 2-transduced dendritic cells enhance the interferon α -induced, CD8⁺ T-cell-dependent immune defense of B16 melanoma. *Cancer Res* 2001;61:8643-6.
- Sutmoller RPM, van Duivenvoorde LM, van Elsas A, Schumacher TNM, Wildenberg ME, Allison JP, et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25⁺ regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* 2001;194:823-32.
- Berendt MJ, North RJ. T-cell-mediated suppression of anti-tumor

- nity. An explanation for progressive growth of an immunogenic tumor. *J Exp Med* 1980;151:69-80.
29. North RJ. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J Exp Med* 1982;155:1063-74.
30. Bursucker I, North RJ. Suppression of generation of concomitant antitumor immunity by passively transferred suppressor T-cells from tumor-bearing donors. *Cancer Immunol Immunother* 1985;19:215-8.
31. Awwad M, North RJ. Sublethal, whole-body ionizing irradiation can be tumor promotive or tumor destructive depending on the stage of development of underlying antitumor immunity. *Cancer Immunol Immunother* 1988;26:55-60.
32. Awwad M, North RJ. Cyclophosphamide-induced immunologically mediated regression of a cyclophosphomide-resistant murine tumor: a consequence of eliminating precursor L3T4+ suppressor T-cells. *Cancer Res* 1989;49:1649-54.
33. Taguchi O, Takahashi T. Administration of anti-interleukin-2 receptor alpha antibody in vivo induces localized autoimmune disease. *Eur J Immunol* 1996;26:1608-12.
34. Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, et al. Cutting Edge: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. *J Immunol* 2002;168:4272-6.
35. Taguchi O, Kontani K, Ikeda H, Kezuka T, Takeuchi M, Takahashi T. Tissue-specific suppressor T-cells involved in self-tolerance are activated extrathymically by self-antigens. *Immunology* 1994;82:365-9.
36. Seddon B, Mason D. Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. *J Exp Med* 1999;189:877-81.
37. McHugh RS, Shevach EM. Cutting edge: depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J Immunol* 2002;168:5979-83.
38. Hall B, Jelbart M, Gurley K, Dorsch S. Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. Mediation of specific suppression by T helper/inducer cells. *J Exp Med* 1985;162:1683-94.
39. Qin S, Cobbold SP, Pope H, Elliott D, Kioussis D, Davies J, et al. "Infectious" transplantation tolerance. *Science* 1993;259:974-7.
40. Waldmann H, Cobbold S. How do monoclonal antibodies induce tolerance? A role for infectious tolerance? *Annu Rev Immunol* 1998;16:619-44.
41. Mohler KM, Streilein JW. Lymphokine production by MLR-reactive reaction lymphocytes obtained from normal mice and mice rendered tolerant of class II MHC antigens. *Transplantation* 1989;47:625-33.
42. Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, et al. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. *J Immunol* 2001;166:3789-96.
43. Kingsley CI, Karim M, Bushell AR, Wood KJ. CD25+CD4+ regulatory T cells prevent graft rejection: CTLA-4 and IL-10-dependent immunoregulation of alloresponses. *J Immunol* 2002;168:1080-6.
44. Sanchez-Fueyo A, Weber M, Domenig C, Strom TB, Zheng XX. Tracking the immunoregulatory mechanisms active during allograft tolerance. *J Immunol* 2002;168:2274-81.
45. Graca L, Cobbold SP, Waldmann H. Identification of regulatory T cells in tolerated allografts. *J Exp Med* 2002;195:1641-6.
46. Taylor PA, Noelle RJ, Blazar BR. CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. *J Exp Med* 2001;193:1311-7.
47. Graca L, Thompson S, Lin C-Y, Adams E, Cobbold SP, Waldmann H. Both CD4+CD25+ and CD4+CD25- regulatory cells mediate dominant transplant tolerance. *J Immunol* 2002;168:5558-65.
48. Suri-Payer E, Cantor H. Differential cytokine requirements for regulation of autoimmune gastritis and colitis by CD4(+)CD25(+) T cells. *J Autoimmun* 2001;16:115-23.
49. McHugh R, Shevach E, Thornton A. Control of organ-specific autoimmunity by immunoregulatory CD4+CD25+ T cells. *Microbes Infect* 2001;3:919-27.
50. Zelenika D, Adams E, Humm S, Graca L, Thompson S, Cobbold SP, et al. Regulatory T cells overexpress a subset of Th2 gene transcripts. *J Immunol* 2002;168:1069-79.
51. McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, et al. CD4+CD25+ Immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 2002;16:311-23.
52. Gavin MA, Clarke SR, Negrou E, Gallegos A, Rudensky A. Homeostasis and anergy of CD4+CD25+ suppressor T cells in vivo. *Nat Immunol* 2002;3:33-41.
53. Dieckmann D, Bruett CH, Ploettner H, Lutz MB, Schuler G. Human CD4+CD25+ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent Type 1-like regulatory T cells. *J Exp Med* 2002;196:247-53.
54. Jonuleit H, Schmitt E, Kakirman H, Stassen M, Knop J, Enk AH. Infectious Tolerance: Human CD25+ regulatory T cells convey suppressor activity to conventional CD4+ T helper cells. *J Exp Med* 2002;196:255-60.
55. Gregori S, Casorati M, Amuchastegui S, Smirardo S, Davalli AM, Adorini L. Regulatory T cells induced by 1a,25-Dihydroxyvitamin D3 and mycophenolate mofetil treatment mediate transplantation tolerance. *J Immunol* 2001;167:1945-53.
56. Zuany-Amorim C, Sawicka E, Manlius C, Le Moine A, Brunet LR, Kemeny DM, et al. Suppression of airway eosinophilia by killed *Mycobacterium vaccae*-induced allergen-specific regulatory T-cells. *Nat Med* 2002;8:625-9.
57. Powrie F, Correaoliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RB(High) and CD45RB(Low) CD4(+) T-cells are important for the balance between protective and pathogenic cell-mediated-immunity. *J Exp Med* 1994;179:589-600.
58. Aranda R, Sydora B, McAllister P, Binder S, Yang H, Targan S, et al. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. *J Immunol* 1997;158:3464-73.
59. Coutinho A, Hori S, Carvalho TL, Caramalho I, Demengeot J. Regulatory T cells: the physiology of autoreactivity in dominant tolerance and "quality control" of immune responses. *Immunol Rev* 2001;182:89-98.
60. Hori S, Carvalho TL, Demengeot J. CD25+CD4+ regulatory T cells suppress CD4+ T cell-mediated pulmonary hyperinflammation driven by *Pneumocystis carinii* in immunodeficient mice. *Eur J Immunol* 2002;32:1282-91.
61. Roths JB, Sidman CL. Both immunity and hyperresponsiveness to *Pneumocystis carinii* result from transfer of CD4+, but not CD8+, T cells into severe combined immunodeficiency mice. *J Clin Invest* 1992;90:673-8.
62. Manabe YC, Bishai WR. Latent *Mycobacterium tuberculosis*-persistence, patience, and winning by waiting. *Nat Med* 2000;6:1327-9.
63. Blankson JN, Persaud D, Siliciano RF. The challenge of viral reservoirs in HIV-1 infection. *Ann Rev Med* 2002;53:557-93.
64. Bogdan C, Gessner A, Solbach W, Rollinghoff M. Invasion, control and persistence of *Leishmania* parasites. *Curr Opin Immunol* 1996;8:517-25.
65. Belkaid Y, Hoffmann KF, Mendez S, Kamhawi S, Udey MC, Wynn TA, et al. The role of interleukin (IL)-10 in the persistence of *Leishmania major* in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. *J Exp Med* 2001;194:1497-506.
66. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ immunoregulatory T lymphocytes control *Leishmania major* persistence and the development of concomitant immunity. *Nature* 2002. In press.
67. Iwashiro M, Messer RJ, Peterson KE, Stromnes IM, Sugie T, Hasenkrug KJ. Immunosuppression by CD4+ regulatory T cells induced by chronic retroviral infection. *Proc Nat Acad Sci U S A* 2001;98:9226-30.
68. MacDonald AJ, Duffy M, Brady MT, McKiernan S, Hall W, Hegarty J, et al. CD4 T helper type 1 and regulatory T cells induced against the same epitopes on the core protein in hepatitis C virus-infected patients. *J Infect Dis* 2002;185:720-7.
69. McGuirk P, McCann C, Mills KHG. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper Type 1 responses by *Bordetella pertussis*. *J Exp Med* 2002;195:221-31.
70. Wills-Karp M. IL-12/IL-13 axis in allergic asthma. *J Allergy Clin Immunol* 2001;107:9-18.
71. Oettgen HC, Geha RS. IgE regulation and roles in asthma pathogenesis. *J Allergy Clin Immunol* 2001;107:429-40.
72. Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399-408.
73. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hypersensitivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;183:195-201.
74. Pope SM, Brandt EB, Mishra A, Hogan SP, Zimmermann N, Matthaei KI, et al. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. *J Allergy Clin Immunol* 2001;108:594-601.

75. Suri-Payer E, Amar AZ, McHugh R, Natarajan K, Margulies DH, Shevach EM. Post-thymectomy autoimmune gastritis: fine specificity and pathogenicity of anti-H/K ATPase-reactive T cells. *Eur J Immunol* 1999;29:669-77.
76. Cottrez F, Hurst SD, Coffman RL, Groux H. T regulatory cells 1 inhibit a Th2-specific response in vivo. *J Immunol* 2000;165:4848-53.
77. Suto A, Nakajima H, Kagami S-I, Suzuki K, Saito Y, Iwamoto I. Role of CD4+CD25+ regulatory T cells in T helper 2 cell-mediated allergic inflammation in the airways. *Am J Respir Crit Care Med* 2001;164:680-7.
78. Shimizu J, Yamazaki S, Takahashi T, Ishida Y, Sakaguchi S. Stimulation of CD25+CD4+ regulatory T cells through GITR breaks immunological self-tolerance. *Nat Immunol* 2002;3:135-42.