Suppressor T Cells in Human Diseases

Clare Baecher-Allan and David A. Hafler

Laboratory of Molecular Immunology, Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Although central and peripheral tolerance are important for the regulation of human immune responses to self- and microbial antigens, an important role of suppressor $CD4^+$ $CD25^+$ T cells is suggested from the recent investigations of human autoimmune diseases and HIV. These new data provide increasing evidence that altered function of $CD4^+$ $CD25^+$ T cells may be an important factor in a wide range of human inflammatory and infectious diseases.

A curious finding in our understanding of human autoimmune diseases has been the presence of self-reactive T cells in the circulation of healthy individuals, as measured by in vitro cloning techniques, without evidence of disease (1). Although central tolerance clearly exerts a major effect in selecting the repertoire of T cells recognizing self-antigens, it is likely that peripheral tolerance is not the sole protector against autoimmune disease. Moreover, although the field of immunology discarded suppressor T cells almost two decades ago, when prevailing views of their mechanism could not stand the scrutiny of molecular biology, it was clear to investigators performing experiments in animal models of autoimmune disease that populations of T cells existed that could be adoptively transferred to prevent the onset of clinical disease.

The past decade has seen the discovery of several T cell populations that appear to have major regulatory effects on T cells responding to both self-antigens and those derived from infectious agents. As is frequently the case, these regulatory T cell populations were discovered first in experimental animal models and subsequently identified in humans. They can broadly be divided into T cells that appear to require antigen-specific, MHC-restricted stimulation, with subsequent secretion of cytokines that down-regulate immune responses—the prototypic Tr1 and Th3 cells, which secrete IL-10 and TGF-β, respectively (2–5)—or more innate regulatory cells that do not appear to require an in vivo "adaptive" immunization to observe their function—the CD1-restricted NKT cells with invariant TCRs, "nonclassical" NK T cells with variant TCRs (6, 7), and the $CD4^+CD25^+$ regulatory T cells (8, 9).

Address correspondence to Clare Baecher-Allan, 77 Ave. Louis Pasteur, Harvard Medical School, Boston, MA 02115. Tel: (617) 525-5330; Fax: (617) 525-5333; email: callan@rics.bwh.harvard.edu

After a decade of investigation in animal models of human disease, a series of recent articles in the *Journal of Experimental Medicine*, including this issue, have placed $CD4^+$ $CD25^+$ T cells as regulators of human immune responses in both autoimmune (10–12) and infectious diseases (13). The potential role of $CD4^+CD25^+$ T cells in human disease presents a striking example of the essential interaction between basic investigation in animal models, both in vivo and in vitro, and clinical investigation of human disease, allowing a greater degree of confidence in the validity of the in vitro observation in humans.

From Mouse Regulatory T Cells to Human. The discovery of $CD4^+CD25^+$ T cells was based on a simple in vivo observation: mouse thymectomy on neonatal day 3 leads to the development of multiorgan autoimmune disease (14). Seminal experiments performed by Sakaguchi and coworkers demonstrated that it was the depletion of $CD4^+CD25^+$ cells that resulted in the onset of systemic autoimmune diseases in these neonatally thymectomized mice (15, 16), and cotransfer of these cells with $CD4^+CD25^-$ cells prevented the development of experimentally induced autoimmune diseases such as colitis, gastritis, insulin-dependent autoimmune diabetes, and thyroiditis (17–23). In sum, these investigations demonstrated that in mice $CD4^+CD25^+$ cells can regulate the responses of autoreactive T cells in vivo. The discovery of the importance of the transcriptional regulator FoxP3 in mouse $CD4^+CD25^+$ T regulatory cell function (24, 25) and the previous observations that patients with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance), a severe inflammatory disease similar to that seen in mice deficient in $CD4^+CD25^+$ regulatory cells, have mutations in *FOXP3* (26), provided a direct correlation between an autoimmune animal model, mouse regulatory T cells, and a human autoimmune disease.

Defining Human $CD4^+CD25^+$ Regulatory T Cells. However, then how could $CD4^+CD25^+$ regulatory T cells be investigated in humans? The in vitro assay developed by Shevach and coworkers (27), where the addition of mouse $CD4^+CD25^+$ T cells to $CD4^+CD25^-$ T cells resulted in suppression of proliferation at ratios of up to ~1:10 suppressor to target T cells, provided a convenient assay of T regulatory function. However, if a human $CD4^+$ T cell population inhibits the proliferation of other T cells in vitro and was isolated solely by expression of CD25, is it

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/2004/03/273/4 $8.00
Volume 200, Number 3, August 2, 2004 273–276
http://www.jem.org/cgi/doi/10.1084/jem.20040812

the same $CD4^+CD25^+$ regulatory T cell population observed in mice where $CD4^+CD25^+$ cells are isolated from animals kept in germ-free facilities with low levels of endogenous T cell activation? Since CD25 can also be expressed by activated T cells, how can we know that the human $CD4^+CD25^+$ regulatory T cell populations now reported by many different groups (9, 28–30) are capable of suppressing immune responses in vivo? Since there is no unique cell surface marker expressed by regulatory T cells, it is difficult to enumerate and analyze these cells.

Heterogeneity of the isolated T regulatory cell populations may explain the contradictory findings as to the mode of action of $CD4^+CD25^+$ T regulatory cells. Human $CD4^+CD25^+$ regulatory T cells have been varyingly reported to suppress by mechanisms that involve either IL-10, or TGF-β, or CTLA4, or none of the above (9, 28, 29, 31–34). During our own initial investigations to determine if suppressive $CD4^+CD25^+$ T cells existed in human peripheral blood, we demonstrated that the separation of $CD4^+$ T cells into $CD25^{neg}$, $CD25^{med}$, and $CD25^{high}$ subsets by FACS® sorting resulted in the segregation of an IL-10–, TGF-β–, and CTLA4-independent but cell contact–dependent suppression only with the $CD25^{high}$ subset (9). Moreover, the $CD25^{high}$ subset was predominantly $CD62L^{high}$ (>95%), a hallmark for resting cells that have not been recently activated. In contrast, the $CD25^{med}$ population exhibits functional variability in vitro: they can either enhance or suppress proliferation in cocultures and they actually contain the majority of recently activated $CD62L^{lo/-}$ $CD4^+$ cells in the circulation. Similar to mouse $CD4^+CD25^+$ cells, these human $CD4^+CD25^{high}$ T cells are unresponsive to in vitro antigenic stimulation (anergic) and strongly suppress the proliferation of responder T cells upon coculture (9).

$CD4^+CD25^+$ T Regulatory Cells in Human Autoimmune Disease. The discovery of methods to evaluate the in vitro function of $CD4^+CD25^+$ T cells in humans has allowed the importance of these regulatory T cells in human immune responses and disease to be examined. As discussed earlier, self-antigen–reactive T cells are present in healthy individuals and in patients with autoimmune disorders. The finding that autoreactive T cells found in patients with autoimmune disease are more easily activated than those from normal subjects (35, 36) leads to the hypothesis that regulatory T cells may normally suppress the activation of self-reactive T cells, and either deficient generation or reduced effector function of these $CD4^+CD25^{high}$ cells plays a role in the development of autoimmunity. Consistent with this hypothesis is the recent work by Danke et al. demonstrating that deletion of $CD4^+CD25^+$ T cells allows marked clonal expansion of human autoreactive T cells in vitro (37).

Multiple sclerosis (MS) is the most common neurologic disease of young adults (38). We compared the frequency and function of $CD4^+CD25^{high}$ T cells derived from patients with MS with those from healthy control subjects. Although we found no change in the frequency of $CD4^+CD25^{high}$ cells, there was a marked decrease in their effector function (10). Using $CD4^+CD25^{high}$ populations that were >98% $CD62L^{high}$, the coculture suppression by patient-derived $CD4^+CD25^{high}$ cells was 20%, whereas the suppression by $CD4^+CD25^{high}$ cells isolated from healthy control subjects was 80%. Differences were also apparent in single cell cloning experiments, in which the cloning frequency of $CD4^+CD25^{high}$ T cells was substantially reduced in patients compared with normal controls. As the strength of signal delivered through the TCR of target T cells is one factor determining whether regulatory $CD4^+CD25^{high}$ T cells can suppress the responder T cell proliferation (39), several different strength stimuli were used to properly examine the function of $CD4^+CD25^{high}$ T cells in controls and in patients with MS. A strong signal provided by maximal concentration of plate-bound anti-CD3 mAb abrogated suppression in both patient and control cocultures. In contrast, using lower concentrations of plate-bound anti-CD3 allowed a defect in the suppressive ability of $CD4^+CD25^{high}$ regulatory cells derived from patients with MS to become apparent. Importantly, comixing experiments demonstrated that the decrease in T cell regulatory function was due to a defect in the $CD4^+CD25^{high}$ T cell subset rather than the possibility that the responder $CD4^+CD25^-$ T cells were refractory to suppression because of the presence of activated autoreactive T cells in the patient samples.

The potential involvement of $CD4^+CD25^{high}$ T cells in human autoimmune polyglandular syndromes (APS), in which several organ-specific autoimmune diseases are clustered, has also been investigated recently (11). There are two variants of APS: type I, which is caused by loss of central tolerance, and type II (APS-II), which is of unknown etiology. As depletion of $CD4^+CD25^+$ regulatory T cells in mice causes a syndrome with multiple endocrinopathies resembling APS-II, Kriegel et al. (11) examined whether defects in peripheral $CD4^+CD25^{high}$ T cells would be found in APS-II. As observed in MS, $CD4^+CD25^{high}$ T cells in patients with APS-II were found at normal frequency but were defective in their suppressive capacity. Furthermore, as in patients with MS the defect was shown not to be due to responder cell resistance, suggesting that the pathogenesis of APS-II is related to the function of these regulatory T cells.

Alterations in regulatory $CD4^+CD25^{high}$ T cells have also been reported in patients with rheumatoid arthritis (40) and in patients with juvenile idiopathic arthritis (41). These studies examined $CD4^+CD25^+$ cells directly at the sites of inflammation and in the peripheral blood, although the potential involvement of IL-10 or TGF-β were not examined. Interestingly, the frequency of $CD4^+CD25^{high}$ T cells was much greater in the synovial fluid compared with the peripheral blood in adult patients with rheumatoid arthritis. Patients with the juvenile disease also exhibited similar increases in synovial fluid $CD4^+CD25^{high}$ cells; however the patients with the more favorable prognostic form of the disease also exhibited an increased frequency of $CD4^+CD25^{high}$ T cells in the peripheral blood. When these regulatory T cells were isolated from the joint and tested in vitro, they demonstrated suppressive activity. As these studies find differences in the frequency of $CD4^+CD25^{high}$ regulatory T cells isolated from the target tissue compared with the peripheral blood, they

suggest that regulatory T cells migrate into the joint and are either inactivated by inflammatory mediators, or alternatively, the enhanced number of regulatory T cells in the synovium may actually initiate the resolution of the episode of immune activity. The observation that the form of JIA with better prognosis correlates with enhanced numbers of peripheral $CD4^+CD25^{high}$ cells is also of interest since it suggests that peripheral expansion of regulatory T cells may occur in this subset of patients. However, the major question remains as to whether regulatory T cells are able to function in the synovial fluid milieu as rheumatologic disease exists in the target tissue in the face of an enhanced number of regulatory T cells that are highly suppressive in vitro.

A new study by Ehrenstein et al. published in this issue (12) further suggests that regulatory T cells may be expanded in vivo in patients with rheumatoid arthritis that respond favorably to treatment with anti-TNFα therapy. Specifically, they demonstrate that the $CD4^+CD25^{high}$ cells isolated from the peripheral blood of patients with active rheumatoid arthritis can suppress the responder CD4 T cell proliferative response but not the secretion of inflammatory cytokine. In contrast, $CD4^+CD25^{high}$ cells isolated from patients after anti-TNFα therapy suppress both proliferation and cytokine secretion. These data may underscore the central role for cytokines in maintaining the inflammatory state in vivo. The authors demonstrated that the ability of bead-isolated $CD4^+CD25^+$ T cells to induce a "suppressive phenotype" after culture with CD4 cells did not occur with regulatory T cells isolated from patients with active disease but did occur in cells isolated after anti-TNFα therapy. Moreover, the suppressive activity was restricted to the $CD25^{high}$ subset compared with $CD4^+CD25^{lo}$ cells. Although this analysis focused on cells in the blood compared with the synovium, the correlation between anti-TNFα responders, $CD4^+CD25^{high}$ frequency, and in vitro suppressor activity suggests that the activity of regulatory T cells in the peripheral blood may reflect what is occurring in the affected joints.

A Role for $CD4^+CD25^+$ T Regulatory Cells in HIV. Also in this issue, the importance of $CD4^+CD25^{high}$ regulatory T cells is extended with a report examining a role for regulatory cells in HIV infection. HIV is associated with loss of $CD4^+$ T cells and progressive $CD4^+$ T cell immune dysfunction, leading to impaired HIV responses early after infection (13). Kinter et al. hypothesized that the basis for the decrease in response to HIV relates to defects in $CD25^+CD4^+$ regulatory T cells. In fact, they observed that in the majority of healthy yet HIV-infected individuals, $CD25^+CD4^{high}$ T cells significantly suppressed cellular proliferation and cytokine production by $CD4^+$ and $CD8^+$ T cells in response to HIV antigens/peptides in vitro. As in patients with MS, these in vitro effects were cell contact dependent and IL-10 and TGF-β independent. Paradoxically, they also found that patients with strong HIV-specific $CD4^+CD25^{high}$ function in vitro had significantly lower levels of plasma viremia and higher $CD4^+$ to $CD8^+$ T cell ratios than patients without regulatory T cell activity. These data suggest that suppression of CD4 T cell activation by regulatory T cells may make HIV replication less favorable, possibly because these cells suppress the activation of $CD4^+$ T cells and thus make fewer target cells available.

Concluding Remarks. It appears that $CD4^+CD25^{high}$ regulatory cells modulate immune responses to self-antigens and infectious agents in humans. The possibility that $CD4^+CD25^+$ regulatory T cells may have expanded in vivo, one interpretation of the observed increases in regulatory T cell frequency in the peripheral blood of patients with JIA and rheumatoid arthritis, indicates that the design of therapies that induce $CD4^+CD25^{high}$ cell expansion may be of clinical relevance. The parallel observations relating $CD4^+CD25^+$ regulatory cells in animal models of autoimmunity and human inflammatory diseases indicate that suppressor cells are back to stay as central regulators of immune responses in human diseases.

We would like to thank Dr. Vijay Kuchroo for helpful discussion and for critically reading the manuscript.

Submitted: 2 July 2004
Accepted: 8 July 2004

References

1. Ota, K., M. Matsui, E.L. Milford, G.A. Mackin, H.L. Weiner, and D.A. Hafler. 1990. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. *Nature*. 346:183–187.
2. Hafler, D.A., S.C. Kent, M.J. Pietrusewicz, S.J. Khoury, H.L. Weiner, and H. Fukaura. 1997. Oral administration of myelin induces antigen-specific TGF-beta 1 secreting T cells in patients with multiple sclerosis. *Ann. NY Acad. Sci.* 835:120–131.
3. Cobbold, S., and H. Waldmann. 1998. Infectious tolerance. *Curr. Opin. Immunol.* 10:518–524.
4. Levings, M.K., and M.G. Roncarolo. 2000. T-regulatory 1 cells: a novel subset of CD4 T cells with immunoregulatory properties. *J. Allergy Clin. Immunol.* 106:S109–S112.
5. Chen, Y., V.K. Kuchroo, J. Inobe, D.A. Hafler, and H.L. Weiner. 1994. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. *Science*. 265:1237–1240.
6. Lehuen, A., O. Lantz, L. Beaudoin, V. Laloux, C. Carnaud, A. Bendelac, J.F. Bach, and R.C. Monteiro. 1998. Overexpression of natural killer T cells protects Valpha14$^-$ Jalpha281 transgenic nonobese diabetic mice against diabetes. *J. Exp. Med.* 188:1831–1839.
7. Milner, J.D., S.C. Kent, T.A. Ashley, S.B. Wilson, J.L. Strominger, and D.A. Hafler. 1999. Differential responses of invariant V alpha 24J alpha Q T cells and MHC class II-restricted CD4+ T cells to dexamethasone. *J. Immunol.* 163:2522–2529.
8. Levings, M.K., R. Sangregorio, and M.G. Roncarolo. 2001. Human CD25(+)CD4(+) T regulatory cells suppress naive and memory T cell proliferation and can be expanded in vitro without loss of function. *J. Exp. Med.* 193:1295–1302.
9. Baecher-Allan, C., J.A. Brown, G.J. Freeman, and D.A. Hafler. 2001. Cd4(+)cd25(high) regulatory cells in human peripheral blood. *J. Immunol.* 167:1245–1253.
10. Viglietta, V., C. Baecher-Allan, H.L. Weiner, and D.A. Hafler. 2004. Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* 199:971–979.
11. Kriegel, M.A., T. Lohmann, C. Gabler, N. Blank, J.R. Kalden,

and H.M. Lorenz. 2004. Defective suppressor function of human $CD4^+$ $CD25^+$ regulatory T cells in autoimmune polyglandular syndrome type II. *J. Exp. Med.* 199:1285–1291.
12. Ehrenstein, M.R., J.G. Evans, A. Singh, S. Moore, G. Warnes, D.A. Isenberg, and C. Mauri. 2004. Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFα therapy. *J. Exp. Med.* 200:277–285.
13. Kinter, A.L., M. Hennessey, A. Bell, S. Kern, Y. Lin, M. Daucher, M. Planta, M. McGlaughlin, R. Jackson, S.F. Zeigler, et al. 2004. $CD25^+CD4^+$ regulatory T cells from the peripheral blood of asympotmatic HIV-infected individuals regulate both $CD4^+$ and $CD8^+$ HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. *J. Exp. Med.* 200:331–343.
14. Kojima, A., and R.T. Prehn. 1981. Genetic susceptibility to post-thymectomy autoimmune diseases in mice. *Immunogenetics.* 14:15–27.
15. Asano, M., M. Toda, N. Sakaguchi, and S. Sakaguchi. 1996. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J. Exp. Med.* 184:387–396.
16. Sakaguchi, S., and N. Sakaguchi. 1989. Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. *J. Immunol.* 142:471–480.
17. McHugh, R.S., and E.M. Shevach. 2002. Cutting edge: depletion of CD4(+)CD25(+) regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J. Immunol.* 168:5979–5983.
18. Mottet, C., H.H. Uhlig, and F. Powrie. 2003. Cutting edge: cure of colitis by CD4(+)CD25(+) regulatory T cells. *J. Immunol.* 170:3939–3943.
19. Salomon, B., D.J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe, and J.A. Bluestone. 2000. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity.* 12:431–440.
20. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. 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.* 155:1151–1164.
21. Read, S., V. Malmstrom, and F. Powrie. 2000. 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.* 192:295–302.
22. Suri-Payer, E., A.Z. Amar, A.M. Thornton, and E.M. Shevach. 1998. 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.* 160:1212–1218.
23. Powrie, F., S. Mauze, and R.L. Coffman. 1997. CD4+ T-cells in the regulation of inflammatory responses in the intestine. *Res. Immunol.* 148:576–581.
24. Fontenot, J.D., M.A. Gavin, and A.Y. Rudensky. 2003. Foxp3 programs the development and function of CD4+ CD25+ regulatory T cells. *Nat. Immunol.* 4:330–336.
25. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 299:1057–1061.
26. Gambineri, E., T.R. Torgerson, and H.D. Ochs. 2003. TI-immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr. Opin. Rheumatol.* 15:430–435.
27. Thornton, A.M., and E.M. Shevach. 1998. $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J. Exp. Med.* 188:287–296.
28. Dieckmann, D., C.H. Bruett, H. Ploettner, M.B. Lutz, and G. Schuler. 2002. Human $CD4^+CD25^+$ regulatory, contact-dependent T cells induce interleukin 10-producing, contact-independent type 1-like regulatory T cells. *J. Exp. Med.* 196: 247–253.
29. Jonuleit, H., E. Schmitt, H. Kakirman, M. Stassen, J. Knop, and A.H. Enk. 2002. Infectious tolerance: human CD25(+) regulatory T cells convey suppressor activity to conventional CD4(+) T helper cells. *J. Exp. Med.* 196:255–260.
30. Walker, M.R., D.J. Kasprowicz, V.H. Gersuk, A. Benard, M. Van Landeghen, J.H. Buckner, and S.F. Ziegler. 2003. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25− T cells. *J. Clin. Invest.* 112:1437–1443.
31. Annunziato, F., L. Cosmi, F. Liotta, E. Lazzeri, R. Manetti, V. Vanini, P. Romagnani, E. Maggi, and S. Romagnani. 2002. Phenotype, localization, and mechanism of suppression of $CD4^+$ $CD25^+$ human thymocytes. *J. Exp. Med.* 196:379–387.
32. Levings, M.K., R. Bacchetta, U. Schulz, and M.G. Roncarolo. 2002. The role of IL-10 and TGF-beta in the differentiation and effector function of T regulatory cells. *Int. Arch. Allergy Immunol.* 129:263–276.
33. Nakamura, K., A. Kitani, and W. Strober. 2001. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. *J. Exp. Med.* 194:629–644.
34. Roncarolo, M.G., S. Gregori, and M. Levings. 2003. Type 1 T regulatory cells and their relationship with CD4+CD25+ T regulatory cells. *Novartis Found. Symp.* 252:115–127; discussion 127–131, 203–110.
35. Scholz, C., K.T. Patton, D.E. Anderson, G.J. Freeman, and D.A. Hafler. 1998. Expansion of autoreactive T cells in multiple sclerosis is independent of exogenous B7 costimulation. *J. Immunol.* 160:1532–1538.
36. Viglietta, V., S.C. Kent, T. Orban, and D.A. Hafler. 2002. GAD65-reactive T cells are activated in patients with autoimmune type 1a diabetes. *J. Clin. Invest.* 109:895–903.
37. Danke, N.A., D.M. Koelle, C. Yee, S. Beheray, and W.W. Kwok. 2004. Autoreactive T cells in healthy individuals. *J. Immunol.* 172:5967–5972.
38. Hafler, D.A. 2004. Multiple sclerosis. *J. Clin. Invest.* 113: 788–794.
39. Baecher-Allan, C., V. Viglietta, and D.A. Hafler. 2002. Inhibition of human CD4(+)CD25(+high) regulatory T cell function. *J. Immunol.* 169:6210–6217.
40. Cao, D., V. Malmstrom, C. Baecher-Allan, D. Hafler, L. Klareskog, and C. Trollmo. 2003. Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. *Eur. J. Immunol.* 33:215–223.
41. Prakken, B.J., R. Samodal, T.D. Le, F. Giannoni, G.P. Yung, J. Scavulli, D. Amox, S. Roord, I. de Kleer, D. Bonnin, et al. 2004. Epitope-specific immunotherapy induces immune deviation of proinflammatory T cells in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* 101:4228–4233.