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Review Article

Regulatory T cells in oral and self-tolerance

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Abstract

Several mechanisms of immunologic tolerance have been proposed, including deletion, anergy and active suppression. Deletion and anergy have been reported based on data from both *in vivo* and *in vitro* experiments, but evidence to support the involvement of active suppression in tolerance has been elusive, principally because of the difficulty in defining regulatory T (Tr) cells with immunosuppressive activity. However, data characterizing the function of Tr cells has recently begun to emerge both from experiments in the field of oral tolerance and from others utilizing transgenic (Tg) mice. One such model which we described uses Tg mice expressing a foreign physiological soluble antigen, beef insulin (BI), while another uses T cell receptor (α/β) Tg mice, whose T cell receptors (TCR) are specific for ovalbumin (OVA). Using such models, adoptive transfer of the Tr clones specific for myelin basic protein (MBP) from orally tolerant mice into naive mice was shown to protect the animals from experimental autoimmune encephalomyelitis (EAE). Transforming growth factor-beta (TGF-β) has been shown to be responsible for the immunosuppression. Tr clones functioning in self-tolerance have also been obtained from mice expressing TCR or BI transgenes. T cell cultures from OVA specific TCR expressing Tg mice were stimulated in the presence of interleukin-10 (IL-10) and OVA peptide to produce Tr clones. The Tr clones secreted IL-10 and TGF-β as immunosuppressive factors. Tr clones with a different characteristic have been obtained from BI Tg mice. Adoptive transfer of the Tr clones into normal mice suppressed the BI specific antibody response. These Tr clones have type II Th cytokine profile and produced TGF-β as an inhibitory cytokine. TGF-β production also led to functional bystander suppression in the BI Tg mice. These three different types of Tr clones show considerable heterogeneity in the cytokine profiles. Further investigation of Tr cells will be important for our understanding of autoimmunity and the development of tactics for the therapy of autoimmune disease.

Key words: active suppression, autoimmunity, interleukin-10, transforming growth factor-beta, transgenic mice.

Introduction

Tolerance is one of the distinguishing features of the immune system, as a consequence of the need to discriminate self and non-self (foreign) antigens. Breakdown of this ability results in pathological immune response to self antigens (autoimmunity). An understanding of the mechanisms of tolerance induction and its maintenance is a prerequisite for the development of therapies for autoimmune disease. These mechanisms are also clinically relevant in transplantation and tumor immunology.

Hyperresponsiveness to antigenic stimuli can be induced both intrathymically (central tolerance) and extrathymically (peripheral tolerance).[1–3] Three main mechanisms to explain tolerance have been proposed. These are clonal

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deletion,[1,4] anergy[5,6] and active suppression.[7] Clonal deletion theory predicts T cell clones which are autoreactive are eliminated by apoptosis.[8] Anergy of T cells develops following incomplete T cell receptor stimulation, resulting in abortive activation of signal transduction.[10] Anergy[5] and clonal deletion[8] have been reported using both *in vitro* and *in vivo* experiments. Clear demonstrations of mechanisms of active suppression have been elusive until recently due to the difficulty of obtaining cloned regulatory T (Tr) cells. Technical advances in establishing transgenic (Tg) mice have made it possible to establish mice expressing 'foreign self antigen', or T cell receptor (TCR), recognizing defined antigen.[11,12] These Tg mice have allowed detailed investigations of molecular mechanisms involved in self-tolerance. As evident from recent reports, active suppression is involved in self-tolerance in these Tg mice.

Other evidence for the existence of Tr cells with suppressive activity has been obtained in the field of oral tolerance.[13] In general, Tr cells involved in oral (and self) tolerance demonstrate a cytokine profile of type II Th cells, although some heterogeneity in the Tr population has become apparent.

In this review, the molecular mechanisms of active suppression using these Tr cells will be discussed based on the results of Tr clones.

ORAL TOLERANCE

Prevention of experimental autoimmune disease

Oral administration of antigen induces systemic hyperresponsiveness to immune response following immunization with the same antigen.[14] Feeding antigen is a classical method used to induce tolerance. Wells first described oral tolerance as a state of abrogation of systemic anaphylaxis caused by immunization of antigen in guinea-pigs.[15] Guinea-pigs immunized with hen egg proteins underwent severe anaphylaxis. This hyperimmune response to the antigen was prevented by previous feeding of the same antigen. Systemic hyperresponsiveness can be observed following the oral administration of a wide variety of antigens such as heterologous red cells, bacteria and viruses.[14] Oral tolerance is observed in both humoral and cellular immune responses. However, T independent antigens are not able to induce hyperresponsiveness. Although B cells may be potentially involved in oral tolerance, this hyperresponsiveness is determined principally by T cells. Oral feeding of hapten-carrier conjugates clearly demonstrated that the development of oral tolerance was carrier dependent.[16,17]

Several reports have confirmed infectivity of oral tolerance. The state of hyperresponsiveness can be transferred to naive animal by adoptive transfer of lymphocytes from antigen-fed animals.[18,19] The findings in adoptive transfer experiments strongly support the hypothesis that Tr cells with immunosuppressive activity are responsible for oral tolerance.[13] Further characterization of the mechanisms of active suppression awaits a critical appraisal of the biological and molecular entity of the suppression.

More recent evidence that oral tolerance could prevent and cure experimental autoimmune disease has revitalized this field.[20–22] Immunization of Lewis rats with myelin basic protein (MBP) induces experimental autoimmune encephalomyelitis (EAE), which is considered an animal model of multiple sclerosis. Oral feeding of the antigen blocks the onset of EAE.[23] Similarly, the feeding of S-antigen, which causes experimental autoimmune uveoretinitis (EAU), prevents the disease.[24] Thus oral tolerance elicited by previous antigen feeding has been convincingly demonstrated in experimental animals.

These experiments have been extended to humans to test if a similar protocol could cancel out immune response following oral administration of antigen. A potent immunogenic protein, keyhole limpet hemocyanin (KLH), was used in experiments on human subjects. The group fed the antigen demonstrated a significant reduction in KLH specific T cell proliferation and delayed skin test, suggesting a protocol of oral feeding could be designed for the therapy of some types of human autoimmune disease.[25]

Mechanism of active suppression in oral tolerance

An important question is which T cell subsets ($CD4^+$ or $CD8^+$ T cells) are responsible for active suppression. Experiments using Lewis rats with MBP have suggested that adoptive transfer of $CD8^+$ T cells was able to protect the naive rats from EAE.[23] However, further experiments with mice clearly demonstrated that both $CD4^+$ and $CD8^+$ T cells possessed suppressive activity.[26] Mice deficient in CD4 or CD8 membrane surface molecules were established by the gene targeting method and used to study oral tolerance. Oral tolerance was induced in mice deficient in CD8 gene expression[27] but in contrast, CD4 depleted or MHC class II deficient mice failed to develop oral tolerance.[28] These results imply that $CD4^+$ Tr cells

were indispensable for the induction of active suppression, although $CD8^+$ Tr cells with suppressive activity can develop.

Recently, interesting mechanisms of oral tolerance have been reported. The dose of antigen fed appears to play an important role in determining the mechanism(s) of tolerance. Large quantities of antigen induce hyperresponsiveness mediated by clonal deletion and anergy, whereas a relatively low dose of antigen favors active suppression.[29,30] The exact molecular events which determine these differences have yet to be elucidated.

Tr cells generated following feeding of a low dose of antigen produce factor(s) inhibitory to immune responses. Transwell cultures of $CD8^+$ T cells indicate that transforming growth factor-beta (TGF-β) is an inhibitory cytokine for active suppression.[31] Anti-TGF-β antibody inhibited active suppression by the supernatant of Tr cells. TGF-β is secreted by a wide variety of cells including NK, macrophages, LAK, B cells, $CD4^+$ T cells and $CD8^+$ T cells. Adoptive transfer of either $CD4^+$ T or $CD8^+$ T cells from SJL mice fed MBP protected recipient animals from EAE.[26] Limiting dilution analysis of T cells from MBP fed mice revealed an increased frequency of TGF-β, interleukin-4 (IL-4) and interleukin-10 (IL-10) producing T cells, whereas the frequency of interferon-γ (IFN-γ) secreting cells is decreased in the same mice.[26] $CD4^+$ T cells showed the dominant production of IL-4 and IL-10 over $CD8^+$ T cells.

Further molecular characterization of the mechanisms of suppression requires cloning of these Tr cells. $CD4^+$ T cells were cloned from bulk cultures derived from mesenteric lymph nodes.[32] Interestingly, TGF-β^{hi} T clones do not secrete IFN-γ or IL-2. Generally speaking, these clones demonstrate a type II Th cell pattern as judged by the cytokine production profile, although the amounts of TGF-β, IL-4 and IL-10 secreted varied considerably from one clone to another. The production of TGF-β seemed to be independent of IL-4 or IL-10. Chen *et al*. proposed to call the Tr subset with TGF-β^{hi} producers type III Th cells, since this population has a cytokine profile different from that of typical type II Th cells.[32]

Three clones were further characterized by this group. Two clones showed secretion of large amounts of TGF-β. One clone secretes an intermediate amount of TGF-β but high amounts of IL-4 and IL-10. The amount of IL-4 production varies from low to intermediate to high. The IL-10 production levels of the three clones were intermediate and high. Adoptive transfer of the three clones protected recipient mice from EAE. One clone (TGF-β^{hi}, IL-4^{int} and IL-10^{int}) in particular was further characterized by *in vivo* experiments. The injection of neutralizing anti-TGF-β antibody blocked the EAE protection activity of the clone. However, the immunosuppressive activity of IL-4 and IL-10 has not been tested *in vivo*. TGF-β is a causative agent for the suppression at least.

SELF TOLERANCE

Tr cells developed *in vitro*

A large body of reports indicate that cytokines play a critical role in the development of functionally different T cell subsets. Differential immune responses can be elicited by the addition of exogenous cytokines.[33] For example, IL-4 and IL-10 produced by type II Th cells obstruct the development of type I Th cells and promote the differentiation of type II Th cells,[34] whereas IFN-γ secreted by type I Th cells inhibits the differentiation of type II Th cells.[35] It is expected that T cell cultures in the presence of particular cytokines may promote the differentiation of some peculiar types of T cells.

Both human and mouse T cell cultures were stimulated *in vitro* in the presence of cytokines to induce a skewed differentiation of T cell.[12] Naive $CD4^+$ T cells were obtained from ovalbumin (OVA) specific αβ T cell receptor (TCR) DO11-10 expressing Tg mice. The T cell cultures were stimulated with antigen presenting splenic cells and OVA peptide in the presence of IL-10, IL-4 + IL-10 or IL-4. The addition of IL-10 or IL-4 + IL-10 prompted the development of T cells secreting IL-10^{hi} and IL-5^{hi}. The production of IL-4 in the cultured cells was minimal. Interestingly, the level of IFN-γ produced was comparable with that of type 0 and type I Th cells. This cytokine production profile is different from that of the typical type II Th cells which generally show a cytokine profile of IFN-γ^{lo} and IL-4^{hi}.

In contrast to these data, cultures with IL-4 developed a typical type II Th subset (IL-4^{hi}, IL-5^{hi}, IL-10^{hi}, IL-2^{hi} and IFN-γ^{hi}). Thus, the addition of IL-10 into the $CD4^+$ T cell cultures generated the development of a unique T cell subset. Groux *et al*. proposed to call these T cells Tr1 cells since the T cell subset demonstrated immunosuppressive activity as described below.[12] Similar Tr1 clones were also generated from human peripheral blood lymphocytes.[12] This human Tr1 subset produced a very high level of IL-10, whereas the secretion level of IL-5, IFN-γ and TGF-β were comparable to those of human type 0 Th cells.

The proliferative response of the Tr1 clones was increased by the addition of neutralizing anti-IL-10 antibody into the cultures. But anti-IL-10 antibody did not

affect growth of type I Th, type II Th or type 0 Th clones. Neutralizing anti-TGF-β antibody also increased the proliferative response of the mouse and human Tr1 clones. Proliferative response of the Tr1 clones was almost completely restored by the combination of anti-TGF-β and IL-10 antibodies. The immunosuppressive activity of the Tr1 clones was tested using naive $CD4^+$ T cells in Transwell cultures. The proliferative responses of both human and mouse $CD4^+$ T cells were inhibited by the Tr1 clones in transwell cultures. Again the combination of the two neutralizing antibodies almost completely restored the growth of $CD4^+$ T cells. These results suggest that both IL-10 and TGF-β are responsible for the immunosuppressive activity of the Tr1 clones.

These Tr1 clones may suppress the proliferation of inflammatory type I Th cells *in vivo*. The function of these Tr1 clones, anti-inflammatory activity, was tested. IL-10 deficient mice developed inflammatory bowel disease (IBD).[36] Treatment with IL-10 considerably mitigated the development of IBD induced by the transfer of $CD4^+$ $CD45RB^{hi}$ T cells into SCID mice.[37] Adoptive transfer of the Tr1 clones also significantly inhibited the disease induced by $CD4^+$ $CD45RB^{hi}$ splenic T cells, though the anti-inflammatory function of the Tr1 clones was demonstrated only when the mice were fed the antigen, OVA.

Tr cells developed in a physiological condition: Transgenic mice expressing beef insulin

We have investigated the mechanisms of self-tolerance to a soluble physiological antigen, insulin.[38,39] To this end we have established Tg BALB/c mice expressing beef insulin (BI). A genomic human insulin gene was mutated *in vitro* to create a BI gene. Insulin consists of A and B chains connected by two interchain disulfide bonds. Amino acid residues (Thr^8/Ile^{10}) of the A chain of the human insulin gene were mutated to Ala^8/Val^{10} of BI.[40] The expression of BI was examined by measuring the production of 'human C-peptide', which is cleaved from pro-insulin to become mature insulin. The transcription of the transgene was regulated in a strict tissue specific manner, such that transgene expression was detected in the β-islet cells of the pancreas but not in other tissues, including the thymus. The basal level of BI was very low (1×10^{-11} to 10^{-10} mol). A glucose shock test increased the BI level approximately fourfold. Thus, BI expression was regulated physiologically.[40]

The BI Tg mice were tolerant to BI and sheep insulin but not to pork insulin, as determined by assay for insulin specific antibody production. T cell receptor repertoire analysis of the Tg mice clearly demonstrated that the TCR repertoire was altered from that of the normal BALB/c mice, which showed restricted TCR repertoire.[41] These results support a hypothesis that T cells expressing TCR with strong affinities to BI were eliminated in the Tg mice, suggesting clonal deletion of T cells specific to BI. Further experiments suggested that the $CD4^+$ T cell population specific for BI contained anergized T cells to BI as well.[40,41]

Active suppression in BI Tg mice

We next examined BI Tg mice to test whether a third mechanism (active suppression) was operational for tolerance.[11] A series of experiments clearly demonstrated that tolerance was induced and maintained extrathymically. Moreover, adoptive transfer experiments revealed that the T cell population could induce suppression of immune response to BI in normal mice. To elucidate the mechanisms of this active suppression, $CD4^+$ T cells responding to BI were cloned from both normal and Tg mice following immunization with BI. Most of the T clones (10/12) from normal mice possessed type I Th cytokine profile (IL-2^{hi}, IFN-γ^{hi}, IL-4^{lo} and IL-10^{lo}), whereas most of the T clones (11/12) derived from the BI Tg mice had a cytokine profile typical of type II Th cells (IL-2^{lo}, IFN-γ^{lo}, IL-4^{hi} and IL-10^{hi}). We examined the immunosuppressive activity of these type II Th clones by adoptive transfer experiments. Four T clones could suppress the antibody response to BI in normal mice. In these experiments, 2×10^6 of the Tr clones were transferred. However, even the transfer of 1×10^7 cells of other type II Th clones lacking suppressive activity did not inhibit antibody response to BI in normal mice (Fig. 1). Adoptive transfer of a decreased number of the 'active' Tr clones (2×10^3) failed to suppress the BI specific antibody response. These results demonstrate that active suppression is functional in self-tolerance to this soluble physiological antigen. Thus, all three mechanisms (deletion, anergy and active suppression) are involved in preventing an autoimmune respose to BI in the BI Tg mice.

We tried to identify immunosuppressive cytokine(s) involved in the BI specific suppression in transwell cultures.[42] The proliferation of a type I Th clone specific for BI was decreased in the presence of the Tr clones.The four Tr clones described above secreted TGF-β at four times the level of the non-suppressive type II Th clones. The addition of neutralizing anti-TGF-β antibody into the transwell cultures restored the proliferation of type I Th clones (Teng *et al*. unpubl. data). However, anti-TGF-β antibody did not show any effect on the proliferation of the Tr clones

themselves, unlike the Tr1 clones described above. Antibodies neutralizing IL-4 and IL-10 did not block the suppression of the proliferative response of type I Th clones, suggesting that these cytokines are not immunosuppressive in the BI Tg mice.

BYSTANDER SUPPRESSION

Tr cells induced by oral tolerance were shown to demonstrate bystander suppression. Tr cells developed by oral administration of antigen suppress the immune response to a third party antigen unrelated to the fed antigen when both the fed antigen and the third party antigen are given simultaneously in the same mice.[43,44] Bystander suppression is generated by immunosuppressive cytokine(s). TGF-β secreted by Tr cells in oral tolerance has been shown to have the bystander suppression function.[44]

The Tr subset in BI Tg mice showed bystander suppression activity as well.[42] This bystander suppression was tested in two different assays (Fig. 2). A Tr clone was adoptively transferred into normal mice and the mice were immunized intraperitoneally with both BI and OVA. Bystander suppression was measured by the decrease of OVA specific antibody response. The antibody response was decreased by some 40% compared to control mice immunized with OVA alone. In the second assay, BI Tg mice were immunized with both BI and OVA and the decrease in OVA specific antibody response was

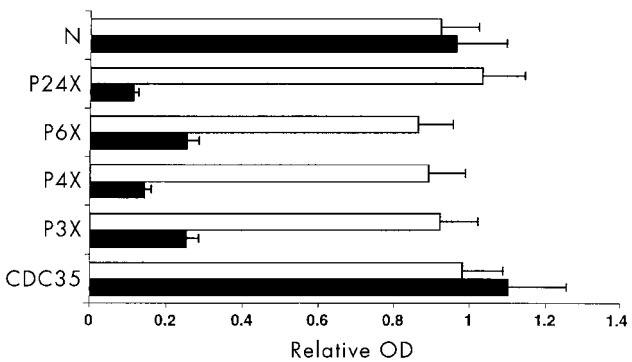

Fig. 1 Beef insulin specific regulatory T clones are able to suppress antibody response after adoptive transfer into syngeneic normal non-transgenic mice. The regulatory T clones (2×10^6 cells of P24X, P6X, P4X and P3X) and CDC35 (control type II T-helper clone) were adoptively transferred into normal BALB/c mice. Two type I T-helper clones were also used as control. These mice were immunized with beef insulin (■) or ovalbumin (□) to measure antibody production to the antigens. N denotes mice without adoptive transfer of T clones. Error bars indicate SD. OD, optical density.

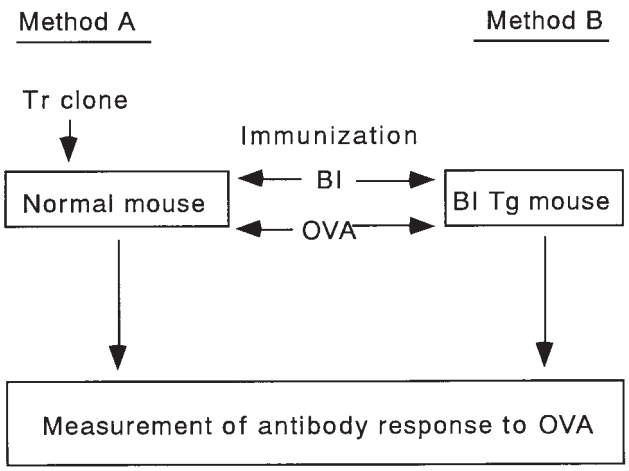

Fig. 2 Two methods to test bystander suppression of beef insulin specific regulatory T cells. Method A, normal mice were adoptively transferred with a regulatory T clone and immunized with beef insulin and ovalbumin. Method B, beef insulin transgenic mice were immunized with beef insulin and ovalbumin. Antibody response to OVA in these mice was compared with that of the mice immunized with ovalbumin alone. OVA, ovalbumin; BI, beef insulin; Tg, transgenic.

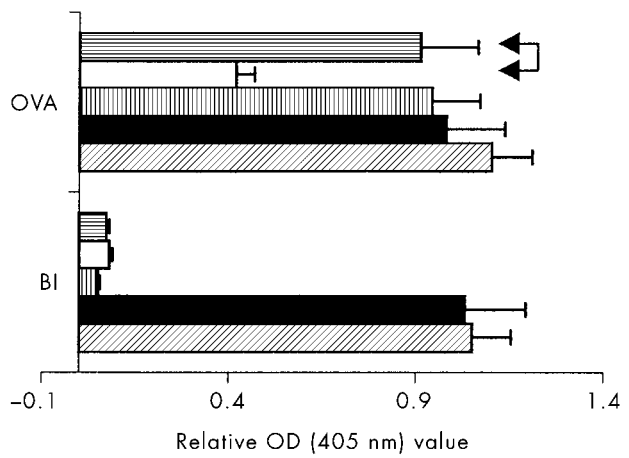

Fig. 3 Bystander suppression is a local response. Beef insulin transgenic mice and normal mice were immunized with ovalbumin and beef insulin in the same (transgenic co-immunization, □) or different (transgenic split-immunization, ▤) site. Serum samples were tested for antibody response to ovalbumin or beef insulin as indicated. (■), normal mice co-immunized with both ovalbumin and beef insulin; (▨), normal mice immunized with beef insulin or ovalbumin alone; (▥) beef insulin transgenic mice immunized with ovalbumin or beef insulin alone. The arrows indicate antibody response in co-immunization and split-immunization. OVA, ovalbumin; BI, beef insulin; OD, optical density.

Table 1. Heterogeneity of Tr clones

Regulatory T clone	IL-2	IFN-γ	IL-4	IL-10	TGF-β	Inhibitory cytokine	References
Myelin basic protein specific	lo	lo	lo/int/hi	int/hi	hi	TGF-β	32
Ovalbumin specific	lo	int/hi	lo	hi	hi	IL-10 TGF-β	12
Beef insulin specific	lo	lo	hi	hi	hi	TGF-β	11, 42

lo, low; int, intermediate; hi, high.

measured. Again the OVA specific antibody response was suppressed by about 40%.

We tested whether TGF-β was responsible for the bystander suppression. The BI Tg mice immunized with BI and OVA were treated with anti-TGF-β antibody. We found that OVA specific antibody response was restored almost to the level of control mice.

Bystander suppression in self-tolerance could be a local reaction rather than a systemic one. Split immunization and co-immunization protocols were performed to test this idea (Fig. 3). In split immunization, the BI Tg mice were immunized separately with BI and OVA at different sites (left and right foot pads). Co-immunization was carried out by immunizing BI and OVA in the same foot pad. The result was clear. Split immunization failed to produce bystander suppression. In contrast, BI Tg mice treated by the co-immunization protocol convincingly demonstrated bystander suppression. Bystander suppression was found to be a temporary phenomenon rather than a long-lasting response. Bystander suppression to OVA specific antibody response in BI Tg mice was measured as a function of days post-immunization. Bystander suppression was observed for the period from day 6–7 to day 13–14 post-immunization. When we tested bystander suppression at 3 months after BI and OVA co-immunization, no bystander suppression for OVA specific antibody responses was detected. Taken together, these experiments suggest that bystander suppression is a local and temporary phenomenon in BI Tg mice.

Conclusion

No subject has been more controversial in modern immunology than active suppression. There is strong evidence for clonal deletion. Neverthless, clones which are autoreactive are found in the periphery.[45] The immune system should be equipped with a device (a fail-safe mechanism) to protect itself from catastrophic self-destruction. It has been hypothesized that active suppression is a mechanism of peripheral tolerance. Conclusive evidence supporting this hypothesis has been elusive for many years, mainly due to the difficulty in defining cellular entity with suppressive activity.

As described in this review, definitive evidence of the involvement of Tr cells in active suppression has now been obtained at the level of T clones. An advanced knowledge of the functions of cytokines in the immune system was a contributing factor in the investigation.

These Tr clones share a common characteristic as far as the growth rate is concerned. The slow proliferation rate of these clones still remains a significant obstacle to further research.

The Tr clones show a diverse cytokine profile (Table 1). Thus, OVA specific Tr1 clones show the production of IFN-γ to levels of typical type I Th. One of the Tr clones developed in oral tolerance showed low IL-4 secretion. Tr1 clones have been obtained from *in vitro* culture in the presence of IL-10. It is not known whether these Tr1 cells function under physiological conditions *in vivo*. IL-10 and TGF-β function as the immunosuppressive factors in the Tr1 clones. In a model transplantation system in mice, where renal and skin graft tolerance can be adoptively transferred by T cell clones produced from portal vein pre-immunized mice, Gorczynski *et al*. reported abolition of activity in adoptive transfer by anti TGF-β and anti IL-10 antibodies.[46]

The BI specific Tr clones secrete TGF-β as an inhibitory factor. IL-10 does not function in the suppression mechanism of the clones.

Further investigation of the molecular mechanisms involved in active suppression will make a significant contribution to our better understanding of autoimmunity and the development of new therapies for the disease.

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REFERENCES

1 Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. *Cell* 1987; 49: 273–80.
2 Webb SR, Morris C, Sprent J. Extra thymic tolerance of mature T cells: Clonal deletion as a consequence of immunity. *Cell* 1990; 63: 1249–56.
3 Blackman MA, Gerhard-Burgert H, Woodland DL. *et al.* A role for clonal inactivation in T cell tolerance to Mls-1^a. *Nature* 1990; 345: 540–2.
4 Kisielow P, Bluthman H, Staertz UD. *et al.* Tolerance in T-cell receptor transgenic mice involves deletion of nonmature $CD4^+$ $CD8^+$ thymocytes. *Nature* 1988; 333: 742–6.
5 Schwarz RH. A cell culture model for T lymphocyte clonal anergy. *Science* 1990; 248: 1349–56.
6 Jenkins MK, Chen LA, Jung G. *et al.* Inhibition of antigen-specific proliferation of type I murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J. Immunol.* 1990; 144: 16–22.
7 Bloom BR, Modlin RL, Salgame P. Stigma variations: Observations on suppressor T cells and leprosy. *Annu. Rev. Immunol.* 1992; 10: 453–88.
8 Rocha B, von Boehmer H. Peripheral selection of the T cell repertoire. *Science* 1991; 251: 1225–8.
9 Quill H. Anergy as a mechanism of peripheral T cell tolerance. *J. Immunol.* 1996; 156: 1325–7.
10 Chan AC, Desai DM, Weiss A. The role of protein tyrosine kinases and protein tyrosine phosphatases in T cell antigen receptor signal transduction. *Annu. Rev. Immunol.* 1994; 12: 555–92.
11 Teng Y-T, Gorczynski RM, Iwasaki S *et al.* Evidence for Th2-like T cell-mediated suppression of antibody responses in transgenic, beef insulin tolerant mice. *Eur. J. Immunol.* 1995; 25: 2522–7.
12 Groux H, O'Garra A, Bigler M. *et al.* A $CD4^+$T-cell subset inhibits antigen specific T-cell responses and prevents colitis. *Nature* 1997; 389: 737–42.
13 Weiner HL, Friedman A, Miller A *et al.* Oral tolerance: Immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annu. Rev. Immunol.* 1994; 12: 809–37.
14 Mowat AM. The regulation of immune-responses to dietary protein antigens. *Immunol. Today* 1987; 8: 93–8.
15 Wells H. Studies on the chemistry of anaphylaxis III. Experiments with isolated proteins, especially those of hen's egg. *J. Infect. Dis*. 1911; 9: 147–51.
16 Titus RG, Chiller JM. Orally induced tolerance: Definition at the cellular level. *Int. Arch. Allergy Immunol.* 1981; 65: 323–8.
17 Richman LK, Chiller JM, Brown WR. *et al.* Enterically induced immunologic tolerance. I. Induction of suppressor T lymphocytes by intragastric administration of soluble proteins. *J. Immunol.* 1978; 121: 2429–34.
18 Challacombe SJ, Tomasi TB. Systemic tolerance and secretory immunity after oral immunization. *J. Exp. Med.* 1980; 152: 1459–72.
19 Mowat AM, Thomas MJ. MacKenzies *et al*. Divergent effects of bacterial lipopolysaccharide on immunity to orally administered protein and particulate antigens in mice. *Immunol*. 1986; 58: 677–83.
20 Thompson HSG, Staines NA. Could specific oral tolerance be a therapy for autoimmune disease? *Immunol. Today* 1990; 11: 396–9.
21 Anderson CN, Bober KA, Robinson MA *et al.* Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc. Natl Acad. Sci. USA* 1986; 83: 7443–6.
22 Higgins PJ, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. *J. Immunol.* 1988; 140: 440–5.
23 Lider O, Santos LM, Lee CSY *et al.* Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and *in vitro* immune responses are mediated by antigen specific $CD8^+$ T lymphocytes. *J. Immunol.* 1989; 142: 748–52.
24 Gregerson DS, Obritsch WF, Donso LA. Oral tolerance in experimental autoimmune uveoretinitis. *J. Immunol.* 1993; 151: 5751–61.
25 Husby S, Mestecky J, Moldoveanu Z. *et al.* Oral tolerance in humans: T cell but not B cell tolerance after antigen feeding. *J. Immunol.* 1994; 152: 4663–70.
26 Chen Y, Inobe J, Weiner HL. Induction of oral tolerance to myelin basic protein in CD8-depleted mice: Both $CD4^+$ and $CD8^+$ cells mediate active suppression. *J. Immunol.* 1995; 155: 910–16.
27 Tada Y, Ho A, Koh DR *et al.* Collagen induced arthritis in CD4 or CD8-deficient mice: $CD8^+$ T cells play a role in initiation and regulate recovery phase of collagen-induced arthritis. *J. Immunol.* 1996; 156: 4520–6.
28 Desvigners C, Bour H, Nicolas J-F *et al.* Lack of oral tolerance but oral priming for contact sensitivity to dinitrofluorobenzene in major histocompatibility complex class II-deficient mice and in $CD4^+$ T cell-depleted mice. *Eur. J. Immunol.* 1996; 26: 1756–61.
29 Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc. Natl Acad. Sci. USA*. 1994; 91: 6688–92.
30 Chen Y, Inobe J, Marks R *et al.* Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* 1995; 376: 177–80.
31 Miller A, Lider O, Roberts AB *et al.* Suppressor T cells generated by oral tolerization to myelin basic protein

suppress both *in vitro* and *in vivo* immune responses by the release of transforming growth factor β after antigen-specific triggering. *Proc. Natl Acad. Sci. USA*. 1992; 89: 421–5.

32 Chen Y, Kuchroo VK, Inobe J. *et al.* Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* 1994; 265: 1237–40.

33 O'Garra A. Cytokines induce the development of functionally heterogenous T helper cell subsets. *Immunity* 1998; 8: 275–83.

34 Sher A, Coffman RL. Regulation of immunity to parasites by T cells and T cell derived cytokines. *Annu. Rev. Immunol.* 1994; 12: 653–73.

35 Fich FW, McKisic MD, Lancki DW *et al.* Differential regulation of murine T lymphocyte subsets. *Annu. Rev. Immunol.* 1993; 11: 29–48.

36 Kuhn R, Lohler J, Rennick W *et al.* Interleukin-10 deficient mice develop chronic enterocolitis. *Cell* 1993; 75: 263–74.

37 Powrie F. Inhibition of Th1 responses prevents inflammatory bowel disease in SCID mice reconstituted with $CD45RB^{hi}$ $CD4^{+}$ cells. *Immunity* 1994; 7: 553–62.

38 Wither J, Pawling J, Phillips L *et al.* Amino acid residues in the T cell receptor CDR3 determine the antigenic reactivity patterns of insulin-reactive hybridomas. *J. Immunol.* 1991; 146: 3513–22.

39 Williams D, Ferguson J, Gariepy J *et al.* Characterization of the insulin A-chain major immunogenic determinant presented by MHC class II $I\text{-}A^{d}$ molecules. *J. Immunol.* 1993; 151: 3627–34.

40 Teng Y-T, Willians DB, Hozumi N *et al.* Multiple levels of regulation for self-tolerance in beef insulin transgenic mice. *Cell. Immunol.* 1996; 173: 183–91.

41 Poplonski L, Vukusic B, Pawling J *et al.* Tolerance is overcome in beef insulin-transgenic mice by activation of low-affinity autoreactive T cells. *Eur. J. Immunol.* 1996; 26: 601–9.

42 Teng Y-T, Gorczynski RM, Hozumi N. The function of TGF-β-mediated innocent bystander suppression associated with physiological self-tolerance *in vivo. Cell Immunol*. (in press).

43 Miller A, Lider O, Weiner HL. Antigen-driven bystander suppression after oral administration of antigens. *J. Exp. Med.* 1991; 174: 791–8.

44 Al-Sabbagh A, Miller A, Santos LMB *et al.* Antigen-driven tissue-specific suppression following oral tolerance: Orally administered myelin basic protein suppresses proteolipid protein-induced experimental autoimmune encephalomyelitis in the SJL mice. *Eur. J. Immunol.* 1994; 24: 2104–9.

45 Blackman M, Kappler J, Marrack P. The role of the T cell receptor in positive and negative selection of developing T cells. *Science* 1990; 248: 1335–41.

46 Gorczynski RM, Chen Z, Zeng H *et al.* Specificity for in vivo graft prolongation in γδ T cell $receptor^{+}$ hybridomas derived from mice given portal vein donor-specific pre-immunization and skin allografts. *J. Immunol.* 1997; 159: 3698–706.