International Reviews of Immunology, 24:159–179, 2005

ISSN: 0883-0185 print/1563-5244 online
DOI: 10.1080/08830180590934949

Taylor & Francis
Taylor & Francis Group

Regulatory T Cells in Autoimmune Diseases: Anti-Ergotypic T Cells

Avishai Mimran
Irun R. Cohen
Department of Immunology,
The Weizmann Institute of Science,
Rehovot, Israel

T regulatory cells play an important role in regulating T-cell responses to self-antigens and control autoimmunity and autoimmune disease. Anti-ergotypic T cells are a subset of such regulatory T cells that respond to activation markers, ergotopes, expressed on other activated T cells. Anti-ergotypic T cells do not respond to nonactivated T cells. Ergotopes include the α-chain of the IL-2 receptor (CD25). Anti-ergotypic T cells were found to downregulate experimental diseases such as experimental autoimmune encephalomyelitis (EAE) and adjuvant arthritis (AA). Anti-ergotypic T cells are present in humans and are activated after T-cell vaccination. Here we review anti-ergotypic T cells in animal models and in humans and contrast anti-ergotypic T cells with other regulatory T-cell subsets.

Keywords: anti-ergotypic, ergotope, T-cell vaccination, regulatory T cells, CD25

INTRODUCTION

The existence of peripheral autoimmune T cells that recognize dominant self-antigens is a property of all healthy immune systems [1,2]. The immunological dominance of self-antigens such as myelin basic protein (MBP), heat-shock protein 60 (HSP60), and insulin is associated with cellular networks consisting of the self-reacting T cells together with a network of regulatory T cells that recognize and respond to the autoimmune T cells. The regulatory T cells are anti-idiotypic and anti-ergotypic T cells [3,4]. Our aim here is to review what is known about the character and functions of anti-ergotypic T cells.

Address correspondence to Prof. Irun R. Cohen, Department of Immunology, The Weizmann Institute of Science, Rehovot, 76100, Israel. E-mail: irun.cohen@weizmann.ac.il

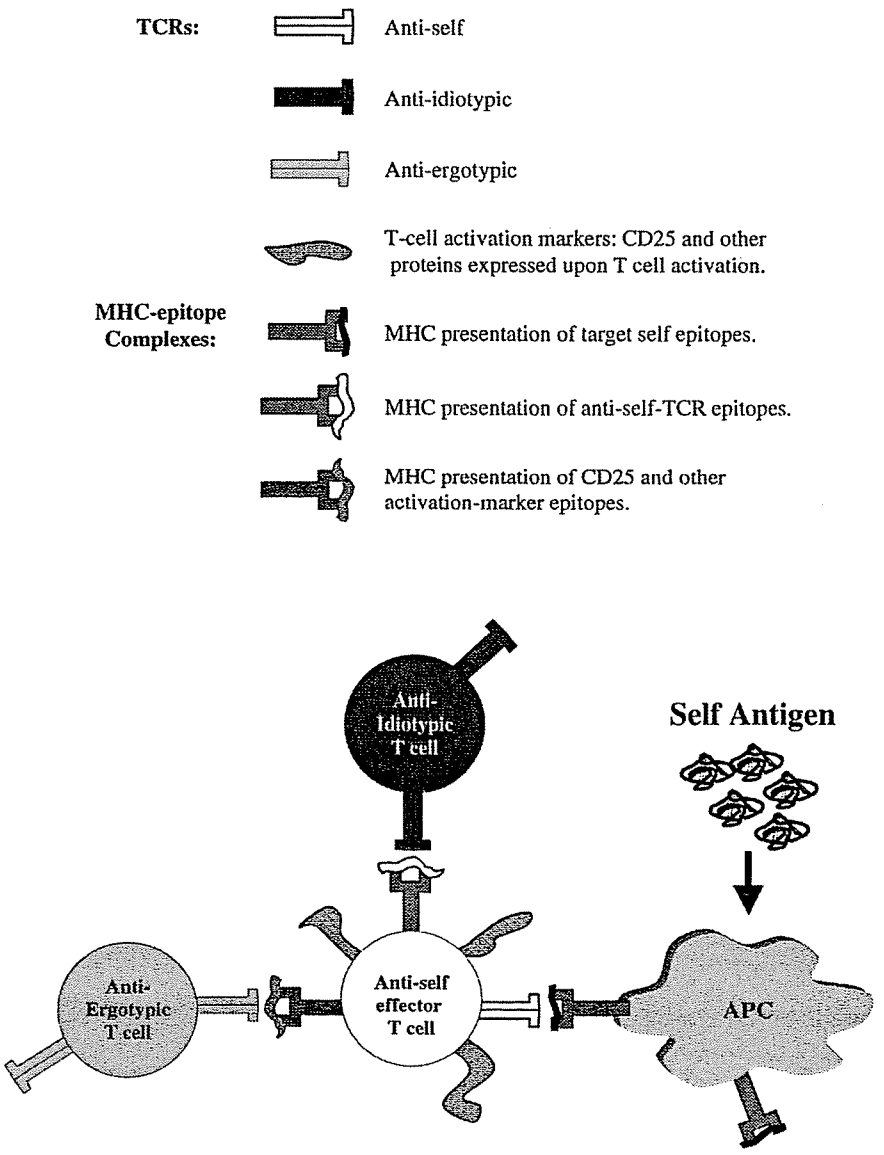

FIGURE 1 A schematic representation of anti-ergotypic and anti-idiotypic T cells and their targets.

Anti-ergotypic regulatory T cells are defined as T cells that respond to activated, syngeneic T cells; anti-ergotypic T cells recognize as antigens the markers of the state of activation, ergotopes, of activated T

cells (Figure 1). Anti-ergotypic T cells do not appear to respond to their target T cells in the resting state. The term *anti-ergotypic T cells* was first coined in the course of studies of T-cell vaccination (TCV) of Lewis rats in the model disease experimental autoimmune encephalomyelitis (EAE). Anti-ergotypic T cells were noted to be generated during the course of TCV immunization in vivo, and the cells were found to be functional; administration of anti-ergotypic T cells to syngeneic rats protected them against EAE [5]. These two observations suggested that anti-ergotypic T cells might fulfill a physiological function in regulating T-cell responses in the immune system.

HISTORY OF ANTI-ERGOTYPIC T CELLS

Preceding the anti-ergotypic concept, T cells responding to activated and not to resting T cells had been described in the beginning of the 1980s in studies of the autologous mixed lymphocyte reaction (AMLR) in humans [6–8]. The AMLR, first defined as the in vitro stimulation of peripheral blood T lymphocytes by autologous non-T lymphocytes, was found to depend on major histocompatibility complex (MHC) class II (HLA-DR) molecules present on the autologous stimulator cells [9]. Later, human T lymphocytes were found to express MHC class II molecules after activation [10], suggesting that activated T cells also might serve as effective stimulators in the AMLR. An early hint of the regulatory function of T cells involved in the classic AMLR was the observation that the AMLR was unaccountably decreased in many clinical autoimmune diseases [11–15].

T cells responding to activated syngeneic T cells were described in the context of experimental autoimmune disease only at the end of the 1980 s, associated with the mechanism of protection induced by TCV. Autoimmune diseases such as EAE [16], adjuvant arthritis [17], thyroiditis [18], collagen II arthritis [19], and experimental autoimmune neuritis [20] can be prevented or treated by administering attenuated but potentially virulent autoimmune T cells specific for the disease-related self-antigens: a procedure called TCV [21,22]. Resistance to disease induced by TCV was associated with anti-idiotypic immunity specific to the autoimmune T-cell receptors [23].

Although the protective effect of TCV was found to be mediated by anti-idiotypic T cells [24], two observations suggested that immunity to T-cell antigen receptors might not be the only protective element induced by TCV. First, TCV was found to work only when the vaccine included T cells activated by specific antigen or a T-cell mitogen. TCV using resting autoimmune T cells could not induce protection [21,22,25]. Second, although a high degree of disease-specific protection is achieved by TCV, a lesser degree of protection also could be

induced by vaccination with nonrelated T cells, provided the T cells had been activated [5]. Lohse and colleagues therefore suggested that rats vaccinated with activated T-cell clones might develop T-cell responses to activation markers expressed on the activated T-cells, in addition to the anti-idiotypic responses targeted to the T-cell receptor (TCR) molecules expressed on the same T cells. T cells responding to activation molecules were termed *anti-ergotypic T cells* (*ergon* in Greek = work, action). Anti-ergotypic T cells thus respond to activated T cells in general, without regard for their idiotypic specificities.

WHAT IS AN ERGOTOPE ?

Anti-ergotypic T cells are able to distinguish between activated and resting T cells. Hence, knowledge of the target structures recognized during the anti-ergotypic T–T cell interaction should be important for understanding the nature of this process and could possibly lead to the development of new therapeutic alternatives for autoimmune diseases. What is the nature of the self-antigens, or ergotopes, recognized by anti-ergotypic T cells? Experiments were done to learn whether anti-ergotypic T cells might be stimulated by secreted factors (cytokines, for example) or by nonsecreted cell components. Secreted factors were unlikely, as supernatants of activated T cells could not induce an anti-ergotypic response [5,26]. The anti-ergotypic response required lysates [5] or protein extracts of activated T cells [26], favoring non-secreted ergotopes. Lohse and colleagues showed that ergotopes appear on activated T cells 12 hours after their activation [26]. Several membranal proteins associated with T-cell activation were suggested as ergotopes. Brod and colleagues reported that adhesion molecules mediate interactions between T cells, only after activation of the stimulator T cells [27]. The high expression levels of LFA-3 and its ligand CD2 upon T-cell activation suggest a potential role of these cell-surface molecules in this T–T cell interaction [27]. Another candidate ergotope was suggested by Ware and associates who showed an increase in MHC class I expression on T cells upon activation [28]. Mor and colleagues implicated the T-cell receptors for IL-2 and TNF-α as ergotopes [29]. T-cell lines raised against immunogenic peptides of TNFR-1 or of the IL-2R α- and β-chains (CD25 and CD122, respectively) could also proliferate in response to activated (but not resting) T cells. Indeed, one of the Mor lines could protect rats from EAE [29]. Thus, a number of protein molecules expressed on the T-cell surface could be ergotopes. The common feature of these proteins is their upregulation upon T-cell activation–essential if they are to serve as ergotopes for the responding anti-ergotypic T cells.

ANTI-ERGOTYPIC T CELLS IN RATS

The Lewis rat was used for studying TCV in EAE [16,23,24], and, as we said above, the identification and characterization of anti-ergotypic T cells were done using this model. Lohse and colleagues showed that lymph node cells (LNCs) could be primed in vivo with clones of syngeneic activated T cells and that the lymph node anti-ergotypic T cells were able to proliferate in vitro when incubated with any syngeneic T cells that had been activated [5]. A similar anti-ergotypic response was also observed in the LNCs of rats that had been primed with complete Freund's adjuvant (CFA) alone. The CFA presumably activated responding T cells in the lymph node, and these activated T cells, in turn, induced an anti-ergotypic response. Anti-ergotypic T cells from such lymph nodes protected Lewis rats from EAE after adoptive transfer [5]. Lohse and associates showed that the anti-ergotypic population included both $CD4^+$ and $CD8^+$ T cells; the $CD8^+$ anti-ergotypic T cells, however, did not lyse in vitro the activated T cells that induced their proliferation [5]. In contrast to the AMLR reaction to activated T cells, the anti-ergotypic response described by Lohse and colleagues was not inhibited by antibodies to MHC class I or class II molecules. Mor and associates reported, however, that rat anti-ergotypic responses were indeed MHC restricted [29].

Based on the observation that anti-ergotypic T-cell lines raised against a peptide of the α-chain of the IL-2 receptor (CD25) could protect rats from EAE [29], we recently undertook to study anti-ergotypic regulation using DNA vaccination of rats with the CD25 gene [30]. We found that DNA vaccination with CD25 could indeed protect rats from adjuvant arthritis (AA). In contrast, DNA vaccination using the CD132 gene (IL-2Rγ) did not protect the rats. CD25 is upregulated during T-cell activation, but CD132 is constitutively expressed [31,32]. Hence CD132, although part of the IL-2 receptor complex, is not an ergotope. We also observed that the LNCs of rats protected from AA by CD25 DNA vaccination proliferated in response to activated syngeneic T cells. We found, in contrast, that the LNC of non-vaccinated control rats undergoing AA did not proliferate in response to activated T cells; thus, anti-ergotypic proliferation was associated with CD25 vaccination and protection against AA. With regard to cytokine secretion, the non-proliferating anti-ergotypic T cells taken from sick rats secreted mainly IFN-γ. In contrast, proliferating anti-ergotypic T cells taken from protected rats secreted mainly IL-10 [30]. Thus, we see that anti-ergotypic T cells may express at least two phenotypes. Anti-ergotypic T cells in rats suffering from AA respond to activated T cells by secreting IFN-γ but do not themselves proliferate.

The function of such anti-ergotypic T cells is yet unknown, but it is conceivable that their secretion of IFN-γ could accelerate and amplify immune responses at their onset.

In contrast to the IFN-γ producers, the anti-ergotypic T cells in rats vaccinated with the ergotope CD25 do proliferate in response to activated T cells and secrete not IFN-γ but IL-10. The CD25-vaccinated rats are protected from developing AA. It remains to be seen whether these two diverse types of anti-ergotypic response reflect two different stages of activation of T cells in a single lineage (naïve and primed) or whether the anti-ergotypic T cells that secrete IFN-γ without proliferating and those that proliferate and secrete IL-10 belong to separate lineages. In either case, the latter phenotype of anti-ergotypic response is associated with control of AA [30].

How does effective anti-ergotypic vaccination regulate the phenotype of the T cells that mediate the arthritis in AA? AA is induced by immunization to antigens of *Mycobacteria* that appear to be cross-reactive with a joint antigen [33]. The target antigen or antigens are present in the mixture of mycobacterial molecules composing purified protein derivative (PPD) [34]. A specific arthritogenic target antigen has been identified in a peptide of the mycobacterial HSP65 molecule; T cells that recognize this peptide were found to mediate AA [35]. Hence, it was conceivable that anti-ergotypic T cells might regulate AA by affecting the behavior of rat T cells responding to these key mycobacterial molecules. It is worthwhile noting that the anti-ergotypic T cells in protected rats did not seem to inhibit the proliferation of the arthritogenic T cells in response to the key mycobacterial antigens. LNCs from CD25-vaccinated rats proliferated well to the HSP65 peptide and they responded to PPD to a higher extent than did LNCs from sham-vaccinated sick rats. The anti-ergotypic vaccination, however, affected the cytokine phenotype of the responding T cells; the T cells in the sick rats secreted mainly IFN-γ and TNF-α in response to the AA antigens, but the T cells in the CD25-vaccinated rats secreted IL-10 and not IFN-γ [30]. IL-10 secreted by the anti-ergotypic T cells could account for protection from AA by deviating arthritogenic T-cell differentiation toward the Th2 class, thereby suppressing disease [36]. This mechanism of protection, however, remains to be proved, and as yet other mechanisms could also account for anti-ergotypic T-cell protection from autoimmune disease.

ANTI-ERGOTYPIC T CELLS IN HUMANS

Since the beginning of the 1990s, clinical trials of TCV, mostly in patients with multiple sclerosis (MS), have generated data about

regulatory anti-ergotypic T cells in humans. Contradictory results have been published by different research groups concerning the phenotype and markers of anti-ergotypic T cells, their interaction with stimulator T cells, their cytokine secretion, and their mechanism of action (Table I). Such contradictions may be due to the different sources of the T cells, whether from healthy individuals, untreated sick patients, or patients treated with T-cell vaccination. As we have seen in rat AA, anti-ergotypic T cells may assume different phenotypes in a dynamic fashion. Different protocols for isolating and studying anti-ergotypic T cells could also generate varied results. Here we shall review the various results and append our comments.

As we mentioned above, human T cells specifically recognizing activated T cells were described more than two decades ago in the human AMLR. The AMLR T–T cell interaction was blocked by anti-MHC-II monoclonal antibody [7,8]. Damle and co-workers showed that AMLR T cells are of the $CD8^+$ phenotype and are able to suppress the proliferation of fresh autologous T cells in vitro [7]. Brod and associates postulated that the human AMLR was similar in many respects to the anti-ergotypic response that had been characterized a year earlier by Lohse and his associates [5]. The Brod group showed that T cells responding to activated T cells could be of the $CD4^+$ or $CD8^+$ phenotypes. They suggested that this T–T cell interaction is contact-dependent and is mediated by CD2–LFA-3 adhesion molecule signaling without a need for antigen-presenting cells (APCs). The response was not blocked by anti-MHC class II monoclonal antibodies. A later study conducted by Bouchonnet and colleagues further characterized the anti-ergotypic T cells proliferating to activated T cells [37]. The responding T cells were found to be of the $CD4^+$ and $CD8^+$ phenotype and expressed TCR-α/β or TCR-γ/δ receptors. They reported that the response did not require APC and was not blocked by anti-MHC monoclonal antibodies. In contrast to earlier studies, they found that the response was not dependent on cell-to-cell contact and could occur across a Transwell membrane. In another experiment, Yuen and associates characterized immunoregulatory $CD8^+$ cells that proliferated in the presence of antigen-activated $CD4^+$ cells in myasthenia gravis patients and healthy donors [38]. The response was blocked by anti-MHC-I monoclonal antibodies. In summary, past research has established the existence of anti-ergotypic T cells in humans and has characterized their proliferation to activated T cells and described their phenotype. However, we know little about the mechanism by which anti-ergotypic T cells regulate their target T cells.

Anti-ergotypic responses in humans have been characterized to a greater degree in TCV clinical trials [39,40]. TCV has made it possible

TABLE I Anti-ergotypic Responses in Humans (See Text for Details)

Year	Reference	Cell source	Regulatory T-cell phenotype				Interaction with activated stimulator T cells		Effects		
			CD4	CD8	α/β	γ/δ	MHC restriction	APC present	Cytokine secretion	Cytotoxicity to T cells	Suppression of T cells
1981	[8]	AMLR (T cells from PBMCs of healthy donors)					+				
1982	[7]	AMLR (T cells from PBMCs of healthy donors)	−	+			+				+
1990	[27]	T-cell clones from PBMCs of healthy donors	+	+			−	−			
1994	[37]	PBMCs or isolated fresh T cells from healthy donors	+	+	+	+	−	−			
1995	[38]	MG patients; healthy controls vaccinated with diphtheria and tetanus toxoids		+			+				
1997	[42]	MS patients, healthy controls, isolating only $CD8^+$ and TCR-γ/δ^+ TCCs		+	+	−	+	+	IFN-γ, TGF-β, TNF-α/β	+	
1997	[42]			−	−	+	−	+	IFN-γ, TNF-α/β	+	
1998	[45]	MS patients after TCV, isolating only TCR-γ/δ^+ TCCs				+			IL-2, TNF-α,IL-10	−	−
2000	[46]	MS patients after TCV, isolating only T lines inhibiting proliferation of targets	+	−			+		IL-4, IL-10	−	+

AMLR, autologous mixed lymphocyte reaction; MHC, major histocompatibility complex; APC, antigen-presenting cell; PMBC, peripheral blood mononuclear cell; MG, myasthenia gravis; MS, multiple sclerosis; TCR, T-cell receptor; TCC, T-cell clone.

to investigate the induction of regulatory T cells responding to the T-cell vaccine and to isolate and propagate these cells in vitro. Although anti-idiotypic T cells responding to the specific T-cell vaccine were the first to be described [40], anti-ergotypic T cells were also found to be induced by TCV in humans [41]. Correale and co-workers isolated from the peripheral blood of MS patients and healthy controls T-cell clones responding to autologous $CD4^+$ clones specific for proteolipid protein (PLP) [42]. These regulatory T-cell clones were of the $CD821^+$–TCR-α/β^+ or TCR-γ/δ^+ ($CD4^-CD8^-$) phenotype. Whereas $CD8^+$–TCR-α/β^+ T cells were either anti-idiotypic (based on recognition of the specific TCR-Vβ of the stimulator clone) or anti-ergotypic (proliferation to autologous antigen-activated T cells), TCR-γ/δ^+ T clones were found to be anti-ergotypic only.

The biological role of TCR-γ/δ^+ cells is poorly understood in general. These cells have been reported to be associated with human organ-specific autoimmune diseases and might have a possible role in regulating TCR-α/β^+ autoreactive T cells [43]. The two anti-ergotypic T-cell populations described by Correale and associates differed in their patterns of recognition and in the mechanisms by which they modulate autoimmune $CD4^+$ T cells: anti-MHC-I monoclonal antibody inhibited the $CD8^+$–TCR-α/β^+ anti-ergotypic T cells but not the TCR-γ/δ^+ anti-ergotypic cells [42]. The cytokine profile of the two anti-ergotypic populations also differed: the $CD8^+$–TCR-α/β^+ cells secreted IFN-γ, TNF-α/β, and TGF-β (and not IL-4 and IL-10), while the TCR-γ/δ^+ cells secreted IFN-γ and TNF-α/β, but not TGF-β. This study showed that $CD8^+$–TCR-α/β^+ anti-ergotypic T cells can mediate regulatory activity either by cytolytic destruction of activated T cells or by the release of specific cytokines. Indeed, IFN-γ secreted by $CD8^+$ cells has been shown to inhibit T-cell responses [44]. Moreover, $CD4^-CD8^-$–TCR-γ/δ^+ anti-ergotypic T cells were found to be able to lyse antigen-activated autologous T-cell clones but not resting T cells [42].

In a later study, Stinissen and associates isolated TCR-γ/δ^+ anti-ergotypic T cells from peripheral blood of MS patients who had been treated with TCV or were untreated [45]. They found that TCV upregulated the TCR-γ/δ^+ T-cell responses to activated autologous T cells. In contrast to the TCR-γ/δ^+ cells obtained from non-vaccinated MS patients of Correale et al. [42], the anti-ergotypic TCR-γ/δ^+ cells from the TCV patients of Stinissen et al. could proliferate to autologous T cells activated by phytohemagglutinin (PHA) and not only to antigen-activated T cells. The phenotype of the TCR-γ/δ^+ T cells obtained from non-vaccinated MS patients was $V\gamma2^+/V\delta2^+$ [42]; but the TCR-γ/δ^+ T cells from the TCV-treated MS patient were predominantly $V\gamma1^+/V\delta1^+$ [45]. In contrast to the TCR-γ/δ^+ T cells obtained from non-vaccinated MS

patients by Correale et al. [42], the TCR-γ/δ^+ T cells from TCV-treated MS patients did not inhibit in vitro proliferation of the stimulator T cells (the vaccine T cells) and displayed only low cytolytic activity toward them [45]. The latter anti-ergotypic T cells, however, produced high levels of IL-2, TNF-α, and IL-10 and low levels of IFN-γ and IL-4. It is likely that these differences in the properties of the TCR-γ/δ^+ anti-ergotypic T-cell populations may be related to the effects of TCV on the MS patients: TCV can upregulate TCR-γ/δ^+responses relative to those of non-vaccinated MS patients or healthy controls. It is also possible that the differences may have been due to individual variation or to differences in cell culture protocols. Our work in the rat model of AA, as we said above, does suggest that anti-ergotypic T cells can change their behavior subsequent to vaccination in vivo [30].

MS patients treated by TCV were studied by Zhang et al. who isolated 104 regulatory T-cell lines from 12 patients [46]. The lines were selected by their ability to inhibit the proliferation of the T-cell clones composing the T-cell vaccine. In this selection, 31% of the isolated lines were of the $CD8^+$ phenotype, and these were found to be anti-idiotypic cytolytic cell lines. The remaining 69% of the regulatory T-cell lines were $CD4^+$ and were found to be anti-ergotypic regulators: they inhibited the proliferation of PHA-activated T cells irrespective of their TCR specificity. These $CD4^+$ anti-ergotypic T cells were not cytolytic but secreted high levels of IL-4 and IL-10. Their reactivity to activated T cells could be blocked by anti-MHC class II antibody.

The above three studies [42,45,46] contribute much to our understanding of the anti-ergotypic T-cell responses in humans. Differences in the results might be explained by the different populations of subjects and by the different methods of T-cell selection: The study of Correale et al. included healthy controls and non-vaccinated MS patients. They selected only $CD8^+$–TCR-α/β^+ and TCR-γ/δ^+ anti-ergotypic T cells and did not study $CD4^+$–TCR-α/β^+ T cells [42]. Stinissen et al. selected only for TCR-γ/δ^+ T cells but only from T-cell vaccinated MS patients [45]. Zang et al. also obtained their T-cell lines from T-cell vaccinated MS patients but selected only the regulatory lines, which inhibited the in vitro proliferation of the immunizing T cells [46]. Clearly, vaccination can modify the differentiation and behavior of anti-ergotypic T cells; more work is needed to understand the whole picture of regulatory anti-ergotypic T cells.

ANTI-ERGOTYPIC T CELLS IN NAÏVE INDIVIDUALS

Because anti-ergotypic T cells have been assumed to be regulators of autoimmunity, the significant studies in the field were done in the

context of autoimmune diseases. TCV was found to induce anti-idiotypic and anti-ergotypic T cell networks, so TCV may represent a means of therapeutic regulation of auto-reactive T cells by taking advantage of pre-existing regulatory networks [1,3,4,22]. If these networks exist prior to the development of autoimmunity, they might have a role in immune regulation in healthy individuals, too. Indeed, anti-ergotypic T cells have been isolated from healthy donors.

The human AMLR experiments using activated T cells as stimulators were done using peripheral blood mononuclear cells (PBMCs) of healthy donors [7,8] and so were the studies of Brod et al. [27] and Bouchonnet et al. [37]. Correale and associates isolated $CD8^+$ anti-ergotypic T cells from the PBMCs of both MS patients and healthy controls [42]. There were no differences in the cytotoxic activity and the cytokine profile between the anti-ergotypic T cells obtained from MS patients and healthy individuals. Yuen and co-workers found anti-ergotypic responses in the PBMCs ($CD8^+$ enriched) of healthy donors and myasthenia gravis patients [38].

Healthy humans are not immunologically naïve. The immune system of any adult has been activated many times during life in response to different antigens. It is conceivable that naturally activated T cells in turn activate anti-ergotypic responses in healthy individuals. Indeed, we have recently detected anti-ergotypic T-cell responses in naïve Lewis rats raised in a specific-pathogen-free animal facility. We found anti-ergotypic responses in primary proliferation experiments using T cells from lymph nodes, spleens, or thymus glands of the naïve rats. This natural anti-ergotypic response was found to be blocked by anti-MHC class II monoclonal antibody, suggesting that the anti-ergotypic reaction is MHC dependent. Depletion of the APCs, in contrast, did not affect anti-ergotypic proliferation (unpublished data). Thus, the anti-ergotypic reactivity in naïve individuals may result from direct T–T interactions. The existence of anti-ergotypic T cells in naïve individuals supports the notion that these T cells could have a physiological role in regulating the immune system and may also take part in the tight regulation of the autoreactive T cells present in the immune system.

ANTI-ERGOTYPIC T CELLS COMPARED TO OTHER REGULATORY T CELLS

The concept of immune regulation by suppressor T cells was advocated by Gershon and Kondo in the beginning of the 1970s [47,48]. However, research in the field reached a dead-end, and the whole

concept of suppression was discredited due to the inability to isolate, sequence, or clone the putative suppressor cells and their factors [49,50]. During the second half of the 1980s, the term *suppression* was replaced by *immune deviation* after the characterization of the Th1 and Th2 T-cell subsets based on their cytokine secretion profile [51,52]. At that time, there was a tendency to attribute regulatory phenomena to the cytokine balance between the Th1/Th2 subsets. In the mid-1990s, Sakaguchi and co-workers described a population of regulatory $CD4^+$ T cells in mice that expressed the IL-2 receptor α-chain (CD25) in the naïve state; this seminal discovery triggered a renewed interest in regulatory T cells generally [53,54]. The Sakaguchi group found that the naturally occurring $CD4^+CD25^+$ suppressor cells control autoreactive T cells and so protect against autoimmune diseases. The $CD4^+CD25^+$ regulators were later detected in humans [55–61].

A variety of regulatory T cells with different phenotypes have been proposed to control self-tolerance and autoimmunity [62–64]. Regulatory T-cell clones were isolated after the induction of oral tolerance and were found to suppress EAE [65]. These clones mediated suppression by way of the secretion of TGF-β and were termed *Th3*. Another method to generate regulatory T-cell clones was to activate T cells in the presence of IL-10. The cytokine profile of these cells is different from that of classical Th1 or Th2 subsets, and the cells were termed *Type 1 T-regulatory cells* (Tr1). Tr1 cells were found to inhibit organ-specific autoimmunity [66–68]. Here, we will review the relationship between anti-ergotypic T cells and the other regulatory T-cell subsets (Table II).

$CD4^+CD25^+$ Suppressors

The CD25 marker, which was traditionally used to identify activated T cells, was found to be constitutively expressed on 5–10% of peripheral T cells, and these T cells exhibit immunoregulatory functions in vitro and in vivo [53]. In contrast to anti-ergotypic T cells, which can be of the $CD4^+$ or $CD8^+$ subsets [27,37,38,46], the $CD25^+$ suppressor population is $CD4^+$ only. $CD4^+CD25^+$ T cells are naturally occurring suppressors, as they differentiate and develop naturally in the thymus [69,70].

We have found anti-ergotypic T cells also to be present in the thymus (manuscript in preparation). The mature fraction of thymic T cells was found to proliferate in response to activated syngeneic T cells, whereas the $CD4^+CD8^+$ immature T-cell fraction did not so

TABLE II Comparison of Different Regulatory T-Cell Populations (See Text for Details)

Property	Regulators		
	Anti-ergotypic	$CD4^+CD25^+$	Tr1
Phenotype/markers	TCR-α/β^+: $CD4^+$, $CD8^+$; and TCR-γ/δ^+	$CD4^+$	$CD4^+$
Naturally occurring/ present in naïve animals	Yes	Yes	?
Is TCR activation required?	Yes	Yes	Yes
Activation by antigen	T-cell activation markers (CD25 and others)	?	Target antigens
Is regulator mechanism antigen specific ?	No	No	No
Is activation APC dependent ?	No	No	Yes
Is regulation contact dependent?	Yes	Yes	No, but maximal with direct contact
Cytokine secretion	IFN-γ, TGF-β, TNF-α/β; IFN-γ, TNF-α/β; TNF-α, IL-10; IL-4, IL-10[a]	Not found to be needed for suppression activity	IL-10; TGF-β; IFN-γ
Mechanism of affecting target T cells	Cytotoxicity; suppression of proliferation; cytokine inhibition[a]	Suppression of proliferation; inhibition of IL-2 transcription	Suppression of proliferation by suppressive cytokines
Proliferation in vitro	Yes	Exogenous IL-2 needed	Exogenous IL-15 needed

[a]Depends on cell phenotype, cell source, and how cells were isolated (see text for details).

respond (unpublished data). Because naïve rat LNCs and human PBMCs contain T cells that proliferate to activated T cells (unpublished data; [38]), we suspect that anti-ergotypic T cells are naturally occurring regulators of immune responses.

$CD4^+CD25^+$ regulators gain suppressive activity after activation via their TCR [71]. However, the antigen or antigens recognized by the $CD4^+CD25^+$ TCR repertoire are still not well defined [71,72]. In contrast to the $CD4^+CD25^+$ suppressors, which are activated but do not proliferate subsequent to TCR signaling [71], anti-ergotypic T cells exhibit high proliferative responses after TCR activation. The $CD4^+CD25^+$ suppressors do not secrete IL-2 by themselves and seem to be dependent on exogenous IL-2 for their generation in vivo [69] and for expansion in vitro [59]. Nevertheless, both $CD4^+CD25^+$ [71] and anti-ergotypic regulators [5], after their activation via the TCR, mediate regulation of autoreactive T cells generally and may inhibit various autoimmune diseases. In both types of regulators, interaction between the regulators and stimulators was found to be contact dependent and APC independent.

As described above, contradictory results were published concerning the mechanisms by which human anti-ergotypic T cells execute their regulatory function and their cytokine secretion profile: cytotoxic effects, secretion of IFN-γ, secretion of IL-10, and secretion of IL-4 and IL-10. In our in vivo studies in rats, we found inhibition of AA without inhibition of the proliferation of the disease effector T cells. The rat anti-ergotypic T cells secreted IL-10, and the autoreactive T cells switched from the Th1 to the Th2 phenotype [30]. In contrast to anti-ergotypic T cells, the $CD4^+CD25^+$ regulators suppress the proliferation of autoreactive $CD4^+CD25^+$ T cells by inhibiting IL-2 transcription [73]. However, the mechanism of this effect is still unknown. $CD4^+CD25^+$ regulators mediated suppression apparently independent of detectable immunosuppressive cytokines [72]. Levings et al. [59] showed that human $CD4^+CD25^+$ cells secrete IL-10 and TGF-β, but neither of these cytokines seemed to be required for the suppressive effect. Nakamura et al. [74] suggested that suppression was mediated via TGF-β bound to the cell surface of the $CD4^+CD25^+$ suppressors. However, genetic studies ruled out any role for TGF-β in the suppressive effect of $CD4^+CD25^+$ cells [72].

To investigate whether the $CD4^+CD25^+$ and anti-ergotypic regulatory populations might be related, we depleted $CD4^+CD25^+$ cells from naïve LNCs and found no loss of anti-ergotypic responses (unpublished data). We conclude that both classes of regulators are independent cell types.

Type 1 T-Regulatory Cells

Both the anti-ergotypic and the $CD4^+CD25^+$ regulators appear to arise naturally. Tr1 regulator cells, in contrast, arise by the activation of $CD4^+$ T cells to specific antigen in the presence of IL-10 [66,68]. Tr1 cells were found to inhibit the induction of inflammatory bowel disease (IBD) in SCID mice when co-transferred with the pathogenic $CD4^+CD45RB^{high}$ T cells [66]. Tr1 cells are antigen specific and can be cloned in vitro merely by the activation of their TCR with specific antigen. Thus, their administration to SCID mice had to be accompanied by the administration of specific antigen to protect against IBD [66]. Like anti-ergotypic and $CD4^+CD25^+$ T cells, Tr1 cells must be activated through their TCR in order to gain suppressive function. Tr1 cells suppress T-cell proliferation, a suppression mediated by a unique cytokine profile: high levels of the immunosuppressive cytokines IL-10 and TGF-β [68,75,76]. Like anti-ergotypic and $CD4^+CD25^+$ regulators, Tr1 cells regulate T-cell responses in an antigen nonspecific manner after their activation [66,68]. The suppressive effect of the Tr1 cells is not contact dependent, as it is mediated by cytokines, in contrast to anti-ergotypic and $CD4^+CD25^+$ regulators, which exert their regulatory effect in a contact-dependent manner. Nevertheless, when Tr1 cells are in direct contact with their targets, their suppression effect is maximal [68].

Unlike anti-ergotypic T cells, Tr1 cells proliferate poorly after activation [66] and do not expand significantly in standard T-cell culture conditions. Poor proliferation could be related to the rapid secretion of IL-10, which might act in an autocrine manner. However, IL-15 restores Tr1 cell proliferation in vitro [77]. This reminds one of the in vitro proliferation of $CD4^+CD25^+$ suppressors, which proliferate only in the presence of exogenous IL-2.

CONCLUSIONS

Despite the contradictory results published about the different subsets of regulatory T cells, the concept of immune regulation and suppression mediated by T cells has finally became accepted by most immunologists. Now that the concept of regulatory cells is no longer taboo, the field may proceed to study important questions related to the differentiation, induction, and function of the different regulatory subsets. Such studies should help us distinguish the regulatory cell types and unravel the relationships between them. Anti-ergotypic T cells are certainly worthy of attention; they are detectable in humans, as well as in experimental animals, they arise in the course of TCV,

and they regulate disease. We would like to know more about the identity of target ergotopes; how diverse a group of molecules are they? Are ergotopes restricted to membranal molecules, or can secreted and intracellular proteins expressed during T-cell activation also serve as regulatory targets? Do anti-ergotypic T cells need co-stimulatory signals; how are they supplied? What are the mechanisms of regulation, and how are they related in the regulatory cascade? There is no end of interesting questions.

Because different regulatory T-cell populations can be generated in vitro and in vivo by different treatments, it is difficult to define which of them occur naturally, and their relevance to the natural maintenance of peripheral tolerance to autoreactive T cells is unclear. An important question concerning regulatory T cells is whether they differentiate in the thymus into a distinct T-cell lineage with specialized regulatory functions. It is also conceivable that the future regulatory T cells emerge from the thymus as naïve T cells but differentiate into regulatory T cells in the periphery upon encounter with antigen. Because anti-ergotypic and $CD4^+CD25^+$ regulatory T cells can be isolated from the peripheral blood of healthy humans and from naïve animal lymph nodes (LNs) and thymuses, these cells might have a role in maintaining immunological nonreactivity to self in the periphery. Both regulators may exert their regulatory function separately or they might cooperate synergistically to keep autoreactive T cells under control. One might suppose that the $CD4^+CD25^+$ T cells act to suppress autoimmune T-cell proliferation, and the anti-ergotypic T cells regulate the autoimmune T cells that evade $CD4^+CD25^+$ suppression. However, considering the dynamic complexity of inflammation [78], it is likely that diverse types of regulators must work together. The immune system, in both its innate and adaptive arms, initiates, mediates, and terminates the varied inflammatory responses that keep the body in shape as well as resistant to infection. Fine-tuning the complexity of inflammation must require the immune system to be complexly regulated within itself [79]. Regulated T-cell autoimmunity has been shown to mediate neuroprotection in the traumatized central nervous system [80]. If this is true for other organs, too, then a regulated measure of autoimmunity may be a useful tool for body maintenance [78]. From this point of view, we may see that regulation is not synonymous with suppression; regulation means proper turning on and not only proper turning off. Perhaps the IFN-γ produced by the naïve ("early") anti-ergotypic response [30] may be an amplifying "on" signal. Speculation aside, it is clear that physiological autoimmunity benefits the body when it is regulated properly; noxious autoimmune disease would seem to emerge from faulty regulation [3].

REFERENCES

[1] I.R. Cohen, The immunological homunculus and autoimmune disease. In N. Talal, (ed.), *Molecular Autoimmunity*, New York: Academic Press, pp. 438–453, 1991.

[2] S. Avrameas, Natural autoantibodies: From 'horror autotoxicus' to 'gnothi seauton', *Immunol. Today*, **12**: 154–159, 1991.

[3] I.R. Cohen and D.B. Young, Autoimmunity, microbial immunity and the immunological homunculus, *Immunol. Today*, **12**: 105–110, 1991.

[4] I.R. Cohen, The cognitive principle challenges clonal selection, *Immunol. Today*, **13**: 441–444, 1992.

[5] A.W. Lohse, F. Mor, N. Karin, and I.R. Cohen, Control of experimental autoimmune encephalomyelitis by T cells responding to activated T cells, *Science*, **244**: 820–822, 1989.

[6] F. Indiveri, B.S. Wilson, C. Russo, V. Quaranta, M.A. Pellegrino, and S. Ferrone, Ia-like antigens on human T lymphocytes: Relationship to other surface markers, role in mixed lymphocyte reactions, and structural profile, *J. Immunol.*, **125**: 2673–2678, 1980.

[7] N.K. Damle and S. Gupta, Autologous mixed lymphocyte reaction in man. V. Functionally and phenotypically distinct human T-cell subpopulations respond to non-T and activated T cells in AMLR, *Scand. J. Immunol.*, **16**: 59–68, 1982.

[8] C. Russo, F. Indiveri, V. Quaranta, G.A. Molinaro, M.A. Pellegrino, and S. Ferrone, Stimulation of human T lymphocytes by PHA-activated autologous T lymphocytes: Analysis of the role of Ia-like antigens with monoclonal antibodies, *Immunogenetics*, **12**: 267–274, 1981.

[9] B. Bergholtz, D. Albrechtsen, and E. Thorsby, Stimulation of T lymphocytes by autologous non-T lymphoid cells. Participation of HLA-D? *Tissue Antigens*, **10**: 27–38, 1977.

[10] H.S. Ko, S.M. Fu, R.J. Winchester, D.T. Yu, and H.G. Kunkel, Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells, *J. Exp. Med.*, **150**: 246–255, 1979.

[11] S. Gupta, S. Fikrig, and E. Orti, Autologous mixed lymphocyte reaction in man. VI. Deficiency of autologous mixed lymphocyte reaction in type I (insulin-dependent) diabetes mellitus, *J. Clin. Lab Immunol.*, **11**: 59–62, 1983.

[12] G.W. Canonica, R. Croci, M. Bagnasco, S. Ferrini, M.E. Cosulich, O. Ferrini, and G. Giordano, Deficiency of the autologous mixed lymphocyte reaction in patients with autoimmune thyroid disease, *Int. Arch. Allergy Appl. Immunol.*, **73**: 137–140, 1984.

[13] M. Bagnasco, G. Ciprandi, A. Orlandini, G. Torre, G.W. Canonica, and G. Giordano, Autologous mixed lymphocyte reaction in Graves' disease: Relationship to clinical status, *Acta Endocrinol. (Copenhagen)*, **110**: 366–372, 1985.

[14] D.A. Hafler, M. Buchsbaum, and H.L. Weiner, Decreased autologous mixed lymphocyte reaction in multiple sclerosis, *J. Neuroimmunol.*, **9**: 339–347, 1985.

[15] R.L. Hirsch, Defective autologous mixed lymphocyte reactivity in multiple sclerosis, *Clin. Exp. Immunol.*, **64**: 107–113, 1986.

[16] A. Ben-Nun, H. Wekerle, and I.R. Cohen, Vaccination against autoimmune encephalomyelitis with T-lymphocyte line cells reactive against myelin basic protein, *Nature*, **292**, 60–61, 1981.

[17] J. Holoshitz, Y. Naparstek, A. Ben-Nun, and I.R. Cohen, Lines of T lymphocytes induce or vaccinate against autoimmune arthritis, *Science*, **219**: 56–58, 1983.

[18] R. Maron, R. Zerubavel, A. Friedman, and I.R. Cohen, T lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice, *J. Immunol.*, **131**: 2316–2322, 1983.

[19] K. Kakimoto, M. Katsuki, T. Hirofuji, H. Iwata, and T. Koga, Isolation of T cell line capable of protecting mice against collagen-induced arthritis, *J. Immunol.*, **140**: 78–83, 1988.

[20] W.A. Taylor and R.A. Hughes, Responsiveness to P2 of blood- and cauda equina-derived lymphocytes in experimental allergic neuritis in the Lewis rat: Preliminary characterisation of a P2-specific cauda equina-derived T cell line, *J. Neuroimmunol.*, **19**: 279–289, 1988.

[21] I.R. Cohen, Regulation of autoimmune disease physiological and therapeutic, *Immunol. Rev.*, **94**: 5–21, 1986.

[22] I.R. Cohen, T-cell vaccination for autoimmune disease: A panorama, *Vaccine*, **20**: 706–710, 2001.

[23] O. Lider, T. Reshef, E. Beraud, A. Ben-Nun, and I.R. Cohen, Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis, *Science*, **239**: 181–183, 1988.

[24] D. Sun, Y. Qin, J. Chluba, J.T. Epplen, and H. Wekerle, Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions, *Nature*, **332**: 843–845, 1988.

[25] Y. Naparstek, A. Ben-Nun, J. Holoshitz, T. Reshef, A. Frenkel, M. Rosenberg, and I.R. Cohen, T lymphocyte lines producing or vaccinating against autoimmune encephalomyelitis (EAE). Functional activation induces peanut agglutinin receptors and accumulation in the brain and thymus of line cells, *Eur. J. Immunol.*, **13**: 418–423, 1983.

[26] A.W. Lohse, T.W. Spahn, T. Wolfel, J. Herkel, I.R. Cohen, and K.H. Meyer zum Buschenfelde, Induction of the anti-ergotypic response, *Int. Immunol.*, **5**: 533–539, 1993.

[27] S.A. Brod, M. Purvee, D. Benjamin, and D.A. Hafler, T-T cell interactions are mediated by adhesion molecules, *Eur. J. Immunol.*, **20**: 2259–2268, 1990.

[28] R. Ware, H. Jiang, N. Braunstein, J. Kent, E. Wiener, B. Pernis, and L. Chess, Human $CD8^+$ T lymphocyte clones specific for T cell receptor V beta families expressed on autologous $CD4^+$ T cells, *Immunity*, **2**: 177–184, 1995.

[29] F. Mor, B. Reizis, I.R. Cohen, and L. Steinman, IL-2 and TNF receptors as targets of regulatory T-T interactions: Isolation and characterization of cytokine receptor-reactive T cell lines in the Lewis rat, *J. Immunol.*, **157**: 4855–4861, 1996.

[30] A. Mimran, F. Mor, P. Carmi, F.J. Quintana, V. Rotter, and I.R. Cohen, DNA vaccination with IL-2 receptor a-chain gene (CD25) induces an anti-ergotypic response and protects Lewis rats from adjuvant arthritis. *J. Clin. Invest.*, **113**: 924–932, 2004.

[31] Y. Minami, T. Kono, T. Miyazaki, and T. Taniguchi, The IL-2 receptor complex: Its structure, function, and target genes, *Annu. Rev. Immunol.*, **11**: 245–268, 1993.

[32] T. Taniguchi, and Y. Minami, The IL-2/IL-2 receptor system: a current overview, *Cell*, **73**: 5–8, 1993.

[33] W. van Eden, J. Holoshitz, Z. Nevo, A. Frenkel, A. Klajman, and T.R. Cohen, Arthritis induced by a T-lymphocyte clone that responds to Mycobacterium tuberculosis and to cartilage proteoglycans, *Proc. Natl. Acad. Sci. U. S. A.*, **82**: 5117–5120, 1985.

[34] X. Paliard, S.G. West, J.A. Lafferty, J.R. Clements, J.W. Kappler, P. Marrack, and B.L. Kotzin, Evidence for the effects of a superantigen in rheumatoid arthritis, *Science*, **253**: 325–329, 1991.

[35] W. van Eden, J.E. Thole, R. van der Zee, A. Noordzij, J.D. van Embden, E.J. Hensen, and I.R. Cohen, Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis, *Nature*, **331**: 171–173, 1988.

[36] A. O'Garra, L. Steinman, and K. Gijbels, $CD4^+$ T-cell subsets in autoimmunity, *Curr. Opin. Immunol.*, **9**: 872–883, 1997.

[37] F. Bouchonnet, D. Lecossier, A. Bellocq, I. Hamy, and A.J. Hance, Activation of T cells by previously activated T cells. HLA-unrestricted alternative pathway that modifies their proliferative potential, *J. Immunol.*, **153**: 1921–1935, 1994.

[38] M.H. Yuen, M.P. Protti, B. Diethelm-Okita, L. Moiola, J.F. Howard, Jr., and B.M. Conti-Fine, Immunoregulatory $CD8^+$ cells recognize antigen-activated $CD4^+$ cells in myasthenia gravis patients and in healthy controls, *J. Immunol.*, **154**: 1508–1520, 1995.

[39] D.A. Hafler, I. Cohen, D.S. Benjamin, and H.L. Weiner, T cell vaccination in multiple sclerosis: A preliminary report, *Clin. Immunol. Immunopathol.*, **62**: 307–313, 1992.

[40] J. Zhang, R. Medaer, P. Stinissen, D. Hafler, and J. Raus, MHC-restricted depletion of human myelin basic protein-reactive T cells by T cell vaccination, *Science*, **261**: 1451–1454, 1993.

[41] A.W. Lohse, N.P. Bakker, E. Hermann, T. Poralla, M. Jonker, and K.H. Meyer zum Buschenfelde, Induction of an anti-vaccine response by T cell vaccination in non-human primates and humans, *J. Autoimmun.*, **6**: 121–130, 1993.

[42] J. Correale, M. Rojany, and L.P. Weiner, Human $CD8^+$ TCR-alpha beta(+) and TCR-gamma delta(+) cells modulate autologous autoreactive neuroantigen-specific $CD4^+$ T-cells by different mechanisms, *J. Neuroimmunol.*, **80**: 47–64, 1997.

[43] S.H. Kaufmann, gamma/delta and other unconventional T lymphocytes: What do they see and what do they do? *Proc. Natl. Acad. Sci. U. S. A.*, **93**: 2272–2279, 1996.

[44] K.E. Balashov, S.J. Khoury, D.A. Hafler, and H.L. Weiner, Inhibition of T cell responses by activated human $CD8^+$ T cells is mediated by interferon-gamma and is defective in chronic progressive multiple sclerosis, *J. Clin. Invest.*, **95**: 2711–2719, 1995.

[45] P. Stinissen, J. Zhang, C. Vandevyver, G. Hermans, and J. Raus, Gamma/delta T cell responses to activated T cells in multiple sclerosis patients induced by T cell vaccination, *J. Neuroimmunol.*, **87**: 94–104, 1998.

[46] Y.C. Zang, J. Hong, M.V. Tejada-Simon, S. Li, V.M. Rivera, J.M. Killian, and J.Z. Zhang, Th2 immune regulation induced by T cell vaccination in patients with multiple sclerosis, *Eur. J. Immunol.*, **30**: 908–913, 2000.

[47] R.K. Gershon and K. Kondo, Cell interactions in the induction of tolerance: The role of thymic lymphocytes, *Immunology*, **18**: 723–737, 1970.

[48] R.K. Gershon and K. Kondo, Infectious immunological tolerance, *Immunology*, **21**: 903–914, 1971.

[49] G. Moller, Do suppressor T cells exist? *Scand. J. Immunol.*, **27**: 247–250, 1988.

[50] D.R. Green and D.R. Webb, Saying the 'S' word in public, *Immunol. Today*, **14**: 523–525, 1993.

[51] T.R. Mosmann, H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman, Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins, *J. Immunol.*, **136**: 2348–2357, 1986.

[52] T.R. Mosmann and R.L. Coffman, TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties, *Annu. Rev. Immunol.*, **7**: 145–173, 1989.

[53] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, 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, 1995.

[54] M. Asano, M. Toda, N. Sakaguchi, and S. Sakaguchi, Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation, *J. Exp. Med.*, **184**: 387–396, 1996.

[55] C. Baecher-Allan, J.A. Brown, G.J. Freeman, and D.A. Hafler, CD4+CD25^{high} regulatory cells in human peripheral blood, *J. Immunol.*, **167**: 1245–1253, 2001.

[56] W.F. Ng, P.J. Duggan, F. Ponchel, G. Matarese, G. Lombardi, A.D. Edwards, J.D. Isaacs, and R.I. Lechler, Human CD4(+)CD25(+) cells: A naturally occurring population of regulatory T cells, *Blood*, **98**: 2736–2744, 2001.

[57] L.A. Stephens, C. Mottet, D. Mason, and F. Powrie, F. Human, CD4(+)CD25(+) thymocytes and peripheral T cells have immune suppressive activity in vitro, *Eur. J. Immunol.*, **31**: 1247–1254, 2001.

[58] L.S. Taams, J. Smith, M.H. Rustin, M. Salmon, L.W. Poulter, and A.N. Akbar, Human anergic/suppressive CD4(+)CD25(+) T cells: A highly differentiated and apoptosis-prone population, *Eur. J. Immunol.*, **31**: 1122–1131, 2001.

[59] M.K. Levings, R. Sangregorio, and M.G. Roncarolo, 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, 2001.

[60] H. Jonuleit, E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A.H. Enk, Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood, *J. Exp. Med.*, **193**: 1285–1294, 2001.

[61] D. Dieckmann, H. Plottner, S. Berchtold, T. Berger, and G. Schuler, Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood, *J. Exp. Med.*, **193**: 1303–1310, 2001.

[62] S. Sakaguchi, Regulatory T cells: key controllers of immunologic self-tolerance. *Cell*, **101**: 455–458, 2000.

[63] E.M. Shevach, Regulatory T cells in autoimmunity. *Annu. Rev. Immunol.*, **18**: 423–449, 2000.

[64] M.G. Roncarolo, and M.K. Levings, The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr. Opin. Immunol.*, **12**: 676–683, 2000.

[65] Y. Chen, V.K. Kuchroo, J. Inobe, D.A. Hafler, and H.L. Weiner, Regulatory T cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis, *Science*, **265**: 1237–1240, 1994.

[66] H. Groux, A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. de Vries, and M.G. Roncarolo, A CD4$^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis, *Nature*, **389**: 737–742, 1997.

[67] M.K. Levings, R. Sangregorio, F. Galbiati, S. Squadrone, R. de Waal Malefyt, and M.G. Roncarolo, IFN-alpha and IL-10 induce the differentiation of human type 1T regulatory cells, *J. Immunol.*, **166**, 5530–5539, 2001.

[68] M.G. Roncarolo, R. Bacchetta, C. Bordignon, S. Narula, and M.K. Levings, Type 1T regulatory cells, *Immunol. Rev.*, **182**: 68–79, 2001.

[69] M. Papiernik, M.L. de Moraes, C. Pontoux, F. Vasseur, and C. Penit, Regulatory CD4 T cells: Expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency, *Int. Immunol.*, **10**: 371–378, 1998.

[70] M. Itoh, T. Takahashi, N. Sakaguchi, Y. Kuniyasu, J. Shimizu, F. Otsuka, and S. Sakaguchi, Thymus and autoimmunity: Production of CD25$^+$CD4$^+$ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance, *J. Immunol.*, **162**: 5317–5326, 1999.

[71] A. Mimran, F. Mor, F.J. Quintana, and I.R. Cohen, Anti-ergotypic T cells in naïve rats, *J. Autoimmun.*, in press, 2005.

[72] A.M. Thornton and E.M. Shevach, Suppressor effector function of $CD4^+CD25^+$ immunoregulatory T cells is antigen nonspecific, *J. Immunol.*, **164**: 183–190, 2000.

[73] E.M. Shevach, $CD4^+CD25^+$ suppressor T cells: More questions than answers, *Nat. Rev. Immunol.*, **2**: 389–400, 2002.

[74] A.M. Thornton and E.M. Shevach, $CD4^+CD25^+$ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production, *J. Exp. Med.*, **188**: 287–296, 1998.

[75] K. Nakamura, A. Kitani, and W. Strober, 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, 2001.

[76] K.W. Moore, R. de Waal Malefyt, R.L. Coffman, and A. O'Garra, Interleukin-10 and the interleukin-10 receptor, *Annu. Rev. Immunol.*, **19**: 683–765, 2001.

[77] J.J. Letterio, and A.B. Roberts, Regulation of immune responses by TGF-beta, *Annu. Rev. Immunol.*, **16**: 137–161, 1998.

[78] R. Bacchetta, C. Sartirana, M.K. Levings, C. Bordignon, S. Narula, and M.G. Roncarolo, Growth and expansion of human T regulatory type 1 cells are independent from TCR activation but require exogenous cytokines, *Eur. J. Immunol.*, **32**: 2237–2245, 2002.

[79] I.R. Cohen, Tending Adam's Garden: Evolving the Cognitive Immune Self. London: Academic Press, 2000.

[80] I.R. Cohen, Discrimination and dialogue in the immune system, *Semin Immunol.*, **12**: 215–219; discussion, 257–344, 2000.

[81] M. Schwartz and I.R. Cohen, Autoimmunity can benefit self-maintenance, *Immunol. Today*, **21**: 265–268, 2000.