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Anti-ergotypic Immunoregulation

F. J. Quintana* & I. R. Cohen†

*Center for Neurologic Diseases, Harvard Medical School, Boston, MA, USA; and †Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

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

Abstract

T-cell vaccination (TCV) controls pathogenic autoimmune T-cell responses via two different regulatory cell populations: anti-idiotypic and anti-ergotypic T cells. Anti-idiotypic T cells recognize clone-specific determinants, like the CDR3 region of the T-cell receptor. Anti-ergotypic T cells recognize antigenic determinants derived from activation markers, which are upregulated by activated T cells, like CD25. In this review, we analyse the different components of the anti-ergotypic response: (1) the target T cells, which can be $CD8^+$ or $CD4^+$ T cells that express TCR$\alpha\beta$ or TCR$\gamma\delta$; (2) the ergotope, which can be a T cell-restricted ergotope not expressed by other cell types or a widely expressed, shared ergotope and (3) the anti-ergotypic T cells, which are detectable in the naive immune system, but whose numbers can be expanded during the induction of an immune response against, or as a result of TCV or specific, anti-ergotypic vaccination. Finally, we discuss possible interactions between anti-ergotypic regulators and other regulatory T cells. We propose that the expression of major histocompatibility complex class II molecules by regulatory $CD4^+CD25^+$ T cells may make possible the cross-regulation of anti-ergotypic and $CD4^+CD25^+$ regulatory T cells, fine-tuning immunoregulation in the mature immune system.

Introduction

The mammalian immune system is characterized by the generation of a large repertoire of B- and T-cell receptors (BCRs and TCRs, respectively) through a complex combinatorial process [1]. This combinatorial process, which is responsible for the generation of BCR and TCR diversity, also leads to the generation of self-reactive clones that could potentially trigger autoimmune disorders. Many of these autoreactive clones are removed from the mature immune repertoire by negative selection [2], but many self-reactive clones are positively selected and populate the healthy repertoire [3–8]. Indeed, it has been proposed that natural autoimmunity to certain self-antigens has a physiological function [9, 10]. In any case, autoimmunity, whether generated by design or by accident, must still be kept under the control of the regulatory activities of different cell types. Moreover, it is reasonable to suppose that even immunity to foreign antigens must be controlled.

Based on their cell surface markers and their profile of cytokine secretion, several cell populations with regulatory activities have been identified: $CD4^+CD25^+$ T cells [11], Th3 cells [12, 13], Tr1 cells [14], Qa-1-restricted $CD8^+$ T cells [15] and NK cells [16] among others. In addition, the study of T-cell vaccination (TCV) as a method to treat autoimmune disorders [17] has led to the identification of two different regulatory populations [18]: anti-idiotypic [19, 20] and anti-ergotypic T cells [21]. Anti-idiotypic responses are directed against antigenic determinants that are clone-specific, exemplified by the CDR3 region of the TCR. Hence, the anti-idiotypic response directed against a myelin basic protein (MBP)-specific clone does not cross-react with a clone that carries a different TCR and therefore shows a different antigen specificity [19]. The anti-ergotypic response, in contrast, recognizes antigenic determinants derived from activation markers (ergotopes), like the CD25 molecule, which are upregulated by activated T cells generally (ergon means work or activity in Greek). Thus, the anti-ergotypic response targets syngeneic activated, but not resting T cells regardless of their specificity. In this study, we will analyse the characteristics of anti-ergotypic regulation [21].

The anti-ergotypic response

The anti-ergotypic response can be analysed based on its three basic components: the target T cell, the ergotope and the anti-ergotypic T cell.

The target T cells

To participate in regulatory anti-ergotypic (or anti-idiotypic) responses, a T cell has to be capable of processing and presenting to anti-ergotypic regulators its ergotopes, usually complexed to major histocompatibility complex class I (MHC-I) and/or class II (MHC-II) molecules. Human and rat T cells do express both MHC-I and MHC-II molecules [22] and the co-stimulatory molecules CD80 and CD86 [23]. Therefore, they can activate MHC-restricted anti-ergotypic (and anti-idiotypic) $CD8^+$ or $CD4^+$ T-cell responses. Mouse T cells, however, do not express MHC-II, but they do express the non-classical MHC-I-Qa molecule. MHC-I-Qa is known to mediate T–T regulatory interactions [24], therefore MHC-I-Qa might mediate anti-ergotypic regulation in the mouse. Notably, the surface expression of MHC-II, CD80 and CD86 is upregulated upon TCR-triggered activation [22, 23]; activated T cells are better antigen-presenting cells (APC) and make for better T-cell vaccines [17]. The increased APC function of activated T cells suggests that the participation in anti-ergotypic regulatory networks is part of the T-cell activation programme, probably to guarantee tight control of the T-cell response induced to all antigens – foreign and self.

Thus, the ability of the target T cell to process self-peptides and efficiently present them in MHC-I and MHC-II molecules conditions its control by anti-ergotypic regulators and shapes the nature of the anti-ergotypic response, irrespective of whether it is mediated by $CD4^+$ or $CD8^+$ cells. Of note, although many anti-ergotypic regulators characterized so far are MHC-I or MHC-II-restricted, non-MHC-restricted anti-ergotypic T cells have also been described; most of them display a $TCR\gamma\delta^+$ phenotype.

The ergotope

By definition, an ergotope is an antigenic determinant derived from an activation marker, a molecule whose level of expression – and ultimately its presentation to anti-ergotypic regulators – is upregulated in the course of T-cell activation. Two different types of ergotopes have been identified so far: T cell-restricted ergotopes and shared ergotopes.

T cell-restricted ergotopes

The α-chain of the IL-2 receptor (CD25) is a source of T cell-restricted ergotopes, because CD25 is only expressed by T cells. Anti-ergotypic T-cell responses to CD25 are well documented. Mor *et al*. reported that vaccination of rats with immunogenic peptides derived from the CD25 sequence inhibits the subsequent induction of experimental autoimmune encephalomyelitis (EAE) triggered with gpMBP [25]. Moreover, a DNA vaccine coding for the full-length CD25 could inhibit the development of adjuvant arthritis (AA) [26]. Control peptides or DNA vaccines derived from the CD132 molecule (the γ-subunit of the IL-2 receptor whose expression levels are not affected by the state of activation of the T cell) did not have a significant effect on EAE or AA progression [25, 26]. The clinical importance of CD25 as a source of a ergotopes has been recently highlighted by the isolation of CD25-specific anti-ergotypic regulatory T cells from multiple sclerosis (MS) patients treated by TCV [27].

Shared ergotopes

Recent data suggest that the 60 kDa heat shock protein (HSP60) is a source of ergotopes targeted by regulatory T–T interactions [28]. HSP60 is an endogenous immunomodulatory molecule that exerts a diverse array of effects on the innate and adaptive arms of the immune system [29, 30]. The detection of HSP60-reactive T cells and antibodies in healthy individuals, and the demonstration that immunity to HSP60 might participate in the pathogenesis and control of arthritis, diabetes and Behcet's disease among other diseases highlights its importance for immune homeostasis [29, 30]. However, more relevant for the present discussion is the finding that HSP60 is upregulated by activated T cells [28]. HSP60-derived epitopes are processed and presented by activated T cells, which can therefore stimulate syngenic HSP60-specific T cells (manuscript submitted for publication). However, HSP60 expression is not restricted to T cells; HSP60 is expressed by many cell types under stress [31]. Thus, HSP60 constitutes an example of a molecule that is widely expressed in the stress response, but can only participate in anti-ergotypic regulation when it is expressed by activated T cells, whose augmented APC function allows it to trigger anti-ergotypic regulators.

Shared ergotopes also provide the vehicle for the extension of anti-ergotypic regulation to non-T cell targets. B cells, for example, upregulate their HSP60 levels upon activation (M. Cohen-Sfady M and I. R. Cohen, personal communication). Thus, HSP60 anti-ergotypic regulators could also target activated B cells to control their expansion or function.

All in all, and regardless of whether a particular molecule is expressed only by T cells or enjoys a more promiscuous pattern of expression, any self-antigen can participate in anti-ergotypic regulatory interactions as long as it fulfills three conditions: (1) the expression levels of the candidate ergotope are upregulated in activated T cells; (2) the candidate ergotope is presented on the surface of the target T cells processed and complexed to MHC molecules and (3) regulatory T cells recognize and can be activated by the putative ergotope. Thus, although

hundreds of molecules might be upregulated upon T-cell activation, be processed by the antigen-presenting machinery and make their way to the cell membrane complexed to MHC molecules, only those that are recognized by regulatory T cells are involved in anti-ergotypic regulatory interactions. Therefore, it is the T-cell repertoire that finally selects which are the ergotopes that will be engaged in T–T regulatory interactions.

The anti-ergotypic T cells

The anti-ergotypic response can be mediated by a heterogeneous group of cells that can change its composition according to the immune state of the individual.

Anti-ergotypic T-cell responses in non-immunized individuals

Anti-ergotypic responses are detectable in the thymus of 1-day-old rats, suggesting that their generation is independent of antigen priming [26]. The anti-ergotypic T cells detectable in newborn rats bear a $CD8^+$ phenotype, and include both $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells. These two populations differed in their profile of cytokine secretion and MHC and co-stimulation requirements for activation: $TCR\alpha\beta^+$ anti-ergotypic cells proliferated but did not secrete detectable cytokines, while $TCR\gamma\delta^+$ anti-ergotypic cells secreted IFNγ and TNFα in response to activated T cells [32]. The response of the $TCR\alpha\beta^+$ $CD8^+$ anti-ergotypic T cells was MHC-I-restricted and B7-CD28-dependent; the response of the $TCR\gamma\delta^+$ anti-ergotypic T cells was B7-CD28-dependent, but was not inhibited by antibodies to classical MHC-I or MHC-II molecules [32].

Anti-ergotypic $CD8^+$ T cells can also be isolated from healthy humans. Of note, these $CD8^+$ anti-ergotypic T cells could only recognize antigen-activated, but not phytohaemagglutinin-activated, autologous $CD4^+$ T cells [33]. $CD8^+$ $TCR\alpha\beta^+$ and $CD8^+$ $TCR\gamma\delta^+$ T cells differed in their MHC requirements and pattern of cytokine secretion: $CD8^+$ $TCR\alpha\beta^+$ cells were MHC-I-restricted and secreted IFNγ, TNFα/β and TGFβ while $CD8^+$ $TCR\gamma\delta^+$ cells were not MHC-I-restricted and secreted IFNγ and TNFα/β, but not TGFβ [33]. Thus, the anti-ergotypic response in non-immunized individuals is driven by $CD8^+$ $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ $CD8^+$ or $CD4^+$ T cells; the $TCR\gamma\delta^+$ cell compartment recognizes its target T cells by a non-MHC-mediated mechanism. The presence of anti-ergotypic T-cell reactivity in non-immunized subjects suggest that this type of regulation is of importance for the control of the healthy immune system under physiological conditions. However, one has to keep in mind that non-immunized healthy rats and humans are not immunologically naive since they exist under the constant stimulation of environmental antigens and commensal microbes.

Anti-ergotypic responses in immunized individuals

The vaccination of rats with complete Freund's adjuvant (CFA) triggers strong anti-ergotyic reactivity [34]. This immunization-induced anti-ergotypic response probably results from a process of self-vaccination with the T-cell clones expanded by the CFA. As we already mentioned, T-cell activation leads to the acquisition of APC function by the activated T cells [28]. Thus, the documentation of anti-ergotypic responses following vaccination with a strong immunogen suggests that anti-ergotypic regulators participate in the resolution phase of an immune response, thereby limiting hyper-immune pathology. Indeed, the anti-ergotypic T cells of naive rats secrete IFNγ, a cytokine that has been recently reported to play two complementary roles in T-cell immunity: it initially boosts the induction of the T-cell response, and later on triggers its resolution [35].

Anti-ergotypic responses triggered by therapeutic vaccination

Several studies suggest that the anti-ergotypic response is depressed in autoimmune disorders. Rats at the peak of the experimental autoimmune disease AA showed decreased anti-ergotypic responses [26]. Similarly, patients suffering from MS showed decreased proliferation to autologous activated T cells [36]. Immunization regimes aimed at strengthening natural anti-ergotypic regulatory networks are therefore expected to be beneficial in the control of autoimmune disorders. Indeed, vaccination with activated T cells of a non-relevant specificity [21, 34], or with defined ergotopes [25, 26] has been shown to control the experimental autoimmune diseases AA and EAE. DNA vaccination with CD25 led to the induction of $CD4^+$ and $CD8^+$ $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ anti-ergotypic T cells that secreted IL-10, but not IFNγ; in contrast, the anti-ergotypic regulators detected in non-immunized rats secrete IFNγ and TNFα but not IL-10 [26]. The $TCR\alpha\beta^+$ anti-ergotypic regulators were MHC-I- and MHC-II-restricted, but the anti-ergotypic interactions mediated by $TCR\gamma\delta^+$ cells were MHC-independent [26].

Most of the human anti-ergotypic responses characterized so far were part of studies designed to analyse the effectiveness of TCV for the treatment of MS. TCV seems to have significant effects on the $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ cell compartments. Stinissen *et al*. described the upregulation of $TCR\gamma\delta^+$-mediated anti-ergotypic responses following TCV [37]. This stimulation of $TCR\gamma\delta^+$ anti-ergotypic activity was also accompanied by a shift in the $TCR\gamma\delta^+$ repertoire from $V\gamma2^+/V\delta2^+$ to $V\gamma1^+/V\delta1^+$, and by the production of high levels of IL-2, TNFα and IL-10 by following stimulation with activated autologous T cells [37].

In addition, Zhang and co-workers studied anti-ergotypic and anti-idiotypic responses following TCV in MS

patients by generating vaccine-reactive $CD4^+$ T-cell lines [27]. The authors describe that all the anti-vaccine reactivity mediated by $CD4^+$ $TCR\alpha\beta^+$ cells was indeed anti-ergotypic and directed against epitopes derived from the CD25 molecule [27]. Under their experimental conditions, none of these lines could be raised from healthy controls [27]. These $CD4^+$ anti-ergotypic regulators were MHC-II-restricted, and produced IL-4 and IL-10 upon stimulation with activated autologous T cells [27].

In conclusion, the above results demonstrate that anti-ergotypic T cells are part of the repertoire of naive individuals, and comprise $CD4^+$ and $CD8^+$ $TCR\gamma\delta^+$ and $TCR\alpha\beta^+$ cells. $TCR\gamma\delta^+$ regulators seem to be MHC-independent. In a healthy immune system, these regulatory populations are activated following the induction of T-cell immunity, and probably contribute to the contraction phase or resolution of the immune response. Anti-ergotypic regulation is depressed in autoimmune disorders, but regulatory populations mediated by $TCR\gamma\delta^+$ or $TCR\alpha\beta^+$ can be boosted by vaccination with activated T cells or defined ergotopes. However, vaccination not only expands pre-existing anti-ergotypic regulators, but also changes their phenotypic characteristics.

Overlap and interactions with other regulatory populations

The definition of anti-ergotypic T cells is based on their reactivity against syngeneic activated, but not resting T cells [28]. This definition puts under the category of anti-ergotypic regulators several groups of cells that differ in their surface markers and cytokine secretion profile. Moreover, this definition does not exclude an overlap between anti-ergotypic regulators and other classes of regulatory T cells. Indeed, recent publications suggest an overlap between human $CD4^+CD25^+$ regulatory T cells and anti-ergotypic regulators.

Sakaguchi identified a population of regulatory $CD4^+$ T cells that is characterized by the expression of high levels of surface CD25 [11]. These $CD4^+CD25^+$ T cells show low proliferative responses upon TCR triggering *in vitro*, and can inhibit the proliferation of other T cells by a contact-dependent mechanism [11]. *In vivo*, $CD4^+CD25^+$ T cells regulate a diverse array of T- and B-cell responses [11]. Regulatory $CD4^+CD25^+$ T cells display a highly self-reactive T-cell repertoire that partially overlaps with that of the pathogenic T cells they control [38, 39]. At the molecular level, the regulatory $CD4^+CD25^+$ T cells were found to express the forkhead transcription factor FoxP3 [40]; however, not all the $CD4^+CD25^+$ T cells have a regulatory phenotype and not all the $FoxP3^+$ T cells are $CD4^+CD25^+$ cells [41].

The first study suggesting an overlap between the anti-ergotypic and the $CD4^+CD25^+$ regulatory T-cell populations was carried out by Vandenbark *et al*., who studied the specificity of the $CD4^+CD25^+$ regulatory T-cell compartment in healthy controls and MS patients [42]. They reported that the $CD4^+CD25^+$ T-cell compartment includes anti-idiotypic clones and clones reactive with TCR CDR2 determinants from the germline V gene repertoire. Although anti-ergotypic reactivity was not directly studied, this study was the first to demonstrate that the human $CD4^+CD25^+$ T-cell compartment includes T-cell regulators involved in T–T interactions.

A recent study by the group of Jingwu Zhang has analysed the contribution of anti-ergotypic regulators to the $CD4^+CD25^+$ regulatory T cells induced in MS patients by TCV [27]. The authors reported that almost all of the $CD4^+$ T-cell reactivity directed against the vaccinating T-cell clone is anti-ergotypic. Moreover, the anti-ergotypic regulators were $CD4^+CD25^+$ T cells that could be classified according to their expression levels of FoxP3 [27]. $CD4^+CD25^+FoxP3^+$ T cells exerted their regulatory activity by the secretion of IL-10; IL-10-blocking antibodies could inhibit the regulatory effects [27]. $CD4^+CD25^+FoxP3^-$ T cells, however, secreted both IFNγ and IL-10, but anti-IL-10 neutralizing antibodies had no effect on the inhibitory activities. Taken together, these results suggest that a fraction of the anti-ergotypic regulators are $CD4^+CD25^+$ T cells. Note that such $CD4^+CD25^+$ anti-ergotypic regulatory T cells were not detected in samples taken from the same patients before TCV [27]. Moreover, the anti-ergotypic response of naive rats is not affected by the depletion of $CD4^+CD25^+$ T cells [32], suggesting that $CD4^+CD25^+$ anti-ergotypic T cells might be expanded by TCV but might not play a leading role in the regulation of the immune response of naive individuals.

An alternative, non-exclusive, look at the relationship between $CD4^+CD25^+$ regulatory T cells and the anti-ergotypic regulators results from the observation made by Baecher-Allan *et al*., who demonstrated that a subpopulation of the $CD4^+CD25^+$ regulatory T cells expresses significant levels of the HLA-DR MHC-II molecule on their surface [43]. The expression of MHC-II molecules correlates with the use of different immunoregulatory mechanisms: regulation by $MHC\text{-}II^+$ $CD4^+CD25^+$ cells is contact-dependent, while $MHC\text{-}II^-$ $CD4^+CD25^+$ cells exert their regulatory activity by contact-dependent and contact-independent (IL-10-mediated) mechanisms [44]. In addition, by upregulating MHC-II-associated ergotopes, regulatory $CD4^+CD25^+$ T cells can participate in anti-ergotypic regulation. It is tempting to speculate that the formation of T–T interactions between anti-ergotypic regulators and $CD4^+CD25^+$ T-cell regulators might lead to the cross-regulation of these two cell populations. This interaction might therefore contribute to the fine-tuning and coordination of different immunoregulatory mechanisms. In other words, a direct interaction between

anti-ergotypic and $CD4^+CD25^+$ regulatory T cells might lead to the mutual regulation of the regulators.

Conclusions

We have summarized and discussed the basic principles of anti-ergotypic immune regulation. Anti-ergotypic regulators are generated in the thymus and do not require activation by external antigens. Anti-ergotypic regulators are naturally expanded during antigen-specific immune responses but can also be boosted by TCV or immunization with defined ergotopes administered as peptides or DNA vaccines. The anti-ergotypic regulatory response is mediated by several different cell populations, among them are the $CD4^+CD25^+$ regulatory T cells. Indeed, $CD4^+CD25^+$ T cells can themselves be targeted by anti-ergotypic regulators as a consequence of their expression of relatively high levels of the MHC-II molecule HLA-DR. Thus, we propose that the cross-talk between the anti-ergotypic and the $CD4^+CD25^+$ regulatory T-cell populations fine-tunes the immunoregulatory mechanisms in an individual, and so regulates the regulators.

Many basic questions related to the biology of anti-ergotypic T cells are still unanswered. Probably the most compelling of them regards the fate of the targeted T cells. These and other questions will guide our steps towards a better understanding of the biology of anti-ergotypic regulatory networks, and might eventually lead to its exploitation in the design of new therapies for autoimmune disorders based on the strengthening of pre-existing immunoregulatory networks.

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