

T cell vaccination: An insight into T cell regulation

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Abstract:

Here we review T cell vaccination (TCV), a cell-based, generic approach to immune regulation. We describe TCV clinical trials in multiple sclerosis and other autoimmune diseases; we analyze the immune regulatory mechanisms activated by TCV; we examine the potential of public T cells as TCV mediators, and we discuss the physiology of immune regulation in the light of TCV.

Introduction to T cell vaccination

T cell vaccination (TCV) was discovered in 1981 as an unexpected outcome of our study of experimental autoimmune encephalomyelitis (EAE) in the Lewis rat, a model of multiple sclerosis (MS). Our initial aim was to isolate lines and clones of T cells specifically reactive to myelin basic protein (MBP) that could adoptively produce EAE in otherwise healthy rats. Indeed, we succeeded in isolating encephalitogenic T cells that could cause clinical EAE within 4 days following intravenous inoculation of a million or more cells. Adoptive transfer of disease required that the transferred T cells be in a state of activation triggered in vitro by culture with specific antigen or a T cell mitogen; inoculation of 50-fold greater numbers of non-activated autoimmune T cells could not produce EAE [1]; EAE was a function of the state of the autoimmune T cells, not of their mere presence in the body. Activated anti-MBP T cells were able to satisfy “Koch’s Postulates” for defining specifically autoimmune T cells as the etiologic agents responsible for EAE [2]. This pure culture methodology has made it possible to study T cell behavior generally – their roles, for example, in immune patho-physiology [3], in detecting new target antigens [4], in characterizing T cell migrations [5, 6], and in probing immune system behavior generally [7]; TCV is only one outcome of this platform technology for isolating functional cultures of T cells.

A century before our T cell investigations, Pasteur, Koch and their colleagues had discovered that the etiologic agents of various infectious diseases, upon attenuation, could be used as *vaccines* to induce resistance to the disease caused by the virulent agent of that disease [8]. The conceptual connection between the pathogenic agents of infection – bacteria or viruses – and the T cell agents of autoimmune disease suggested the fanciful idea that, just as attenuated pathogens could vaccinate individuals against infection, attenuated autoimmune T cells might serve to vaccinate individuals against an autoimmune disease.

Experimenting with this idea, we found that resistance to EAE could be induced by injecting rats with encephalitogenic T cell lines or clones whose virulence had been attenuated by irradiation or chemical cross-linkers [9]. We termed this vaccination procedure TCV. As a result of TCV, experimental animals acquired resistance to EAE produced by administering the virulent T cells, but TCV also induced resistance to EAE actively induced by immunization to MBP. Later, we found that resistance to EAE could also be induced by injecting rats with activated, intact anti-MBP T cells; resistance without clinical EAE was obtained by injecting numbers of unattenuated T cells lower than the number needed to cause clinically overt EAE [10]. Thus, resistance or disease was an outcome of the numbers as well as the state of the autoimmune T cells.

We found that TCV was effective in preventing or treating various other model autoimmune diseases: adjuvant arthritis in rats [11], and thyroiditis [12], lupus [13, 14], and spontaneous type 1 diabetes [15] in mice. We also found that TCV using specific allo-reactive T cells could inhibit allograft rejection [16]. Further studies emerging from the initial discovery of TCV have proceeded in three directions: clinical trials of TCV in human autoimmune diseases; characterization of the regulatory mechanisms induced by TCV; and identification of the target T cell molecules that induce TCV. We shall summarize here some of the findings generated by these studies, present new observations, and draw some conclusions about immune regulation in the light of TCV.

TCV clinical trials

The first trials of TCV to treat human autoimmune disease were undertaken within a decade after the first reports of TCV in experimental animals [17, 18], but only now, after two more decades of encouraging clinical reports, is TCV being developed by a pharmaceutical entity for the treatment of MS [19]; the initial results have earned TCV fast-track status by the FDA [20]. One might wonder how it is that a promising, effective, remarkably safe and relatively low-cost form of treatment for serious illness has tarried so long in its clinical translation – the development of new therapies is a complicated business indeed.

In any case, clinical trials of TCV to treat MS have been undertaken in the USA, Europe and Israel; these trials have used autologous T cells isolated from the patient’s peripheral blood and selected by their reactivity to myelin antigens. The TCV trials have demonstrated significant safety – in marked contrast to the undesirable side effects that may accompany the use of biologic agents and immune suppression [21]. The effects of TCV on the manifestations of MS have been generally beneficial; these trials have included double-blinded, placebo controlled studies [22] and the arrest of disease progression in subjects who had failed earlier to respond to standard immune suppression therapy [23]. The clinical studies of TCV in MS

have been reviewed over the years [24-26] and recently [27], and interested persons may consult these reviews for specific information.

Some clinical results of TCV in rheumatoid arthritis [18, 28-31] and in lupus [32] have been reported; the trials have been relatively small and were not placebo controlled; but the results in these diseases too are encouraging. Cell-based, personalized therapies for complex diseases are gaining acceptance, and TCV, as we learn more about how to use it clinically, might well be of benefit to many people.

General immune regulatory mechanisms uncovered by TCV

TCV provides an insight into the general physiology of immune regulation, we believe, because TCV activates the immune system to regulate itself; in contrast to injecting the subject with a preformed, pharmaceutically designed monoclonal antibody to this or that cytokine or receptor molecule, TCV queries the immune system itself: what do you do, immune system, when you are confronted with a population of syngeneic, activated T cells?

Historically, we explored TCV in the context of autoimmune conditions, but we now know that TCV can activate immune regulation even when using T cell vaccines directed to foreign molecules [33, 34]. This implies that T cell activation, resulting from either self-antigens or foreign antigens, can induce endogenous regulatory mechanisms. Autoimmune disease is only a special case of a general phenomenon – any perturbation of a complex system like the immune system requires resolution: what goes up must come down. Immune reactions to any antigen need to be regulated – unnecessary or persisting immune inflammation in itself constitutes a disease. TCV shows us that the immune system can regulate inflammation generally.

The T cell receptor (TCR) is a major inducer of TCV regulation

TCV activates four types of regulatory mediators: Anti-idiotypic, anti-ergotypic, Qa1-restricted CD8+ suppressor T cells, and anti-TCR antibodies (Figure 1) – at least as defined by variously different experimental protocols. These four mechanisms are neither mutually exclusive nor exhaustive: some of these reports may merely reflect different expressions of a shared mechanism and additional regulatory mechanisms may yet be discovered. But these four mechanisms do provide useful insights into immune system regulation. Note that all four mechanisms recognize the TCR, among other molecules expressed by activated T cells (see Figure 1). We shall begin our discussion with the TCR as a target of TCV.

TCV Regulatory Mechanisms

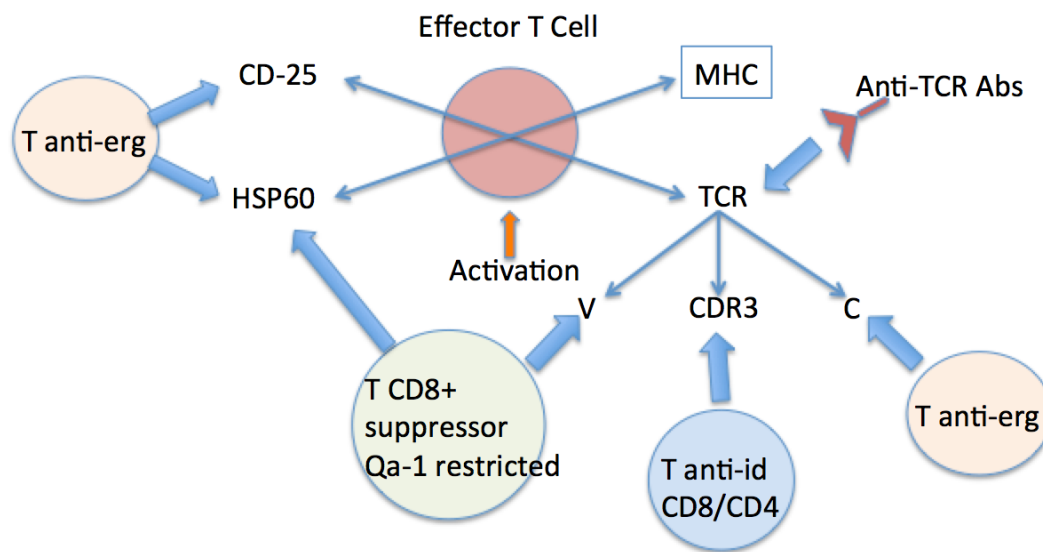


Figure 1. TCV regulatory mechanisms. Activation of autoimmune (and other) effector T cells endows them with the capacity to mount pro-inflammatory immune reactions, but activation also induces the effector T cells to up-regulate their MHC molecules and present signals to regulatory T cells such as TCR idiotopes and ergotopes and ergotopes that include HSP60 and CD-25 epitopes; these signals invite regulation by a variety of anti-id, anti-erg and antibody regulators.

The TCR is composed of three different functional domains encoded by separate genetic elements: the C (constant) domain, which is critical to transducing the antigen-recognition signal into the cell; the V (variable) domain, which contains Complementarity Determining Regions (CDR1 and CDR2) that mainly interact with the MHC molecules of the antigen-presenting cell (APC), and so determine MHC restriction; and the hyper-variable CDR3 region formed by VDJ recombination, which interacts directly with the antigen epitope presented by the MHC. Investigation of the regulatory response to TCV has uncovered T cell regulatory mechanisms targeted to each of the three TCR domains, along with anti-TCR antibodies (Figure 2).

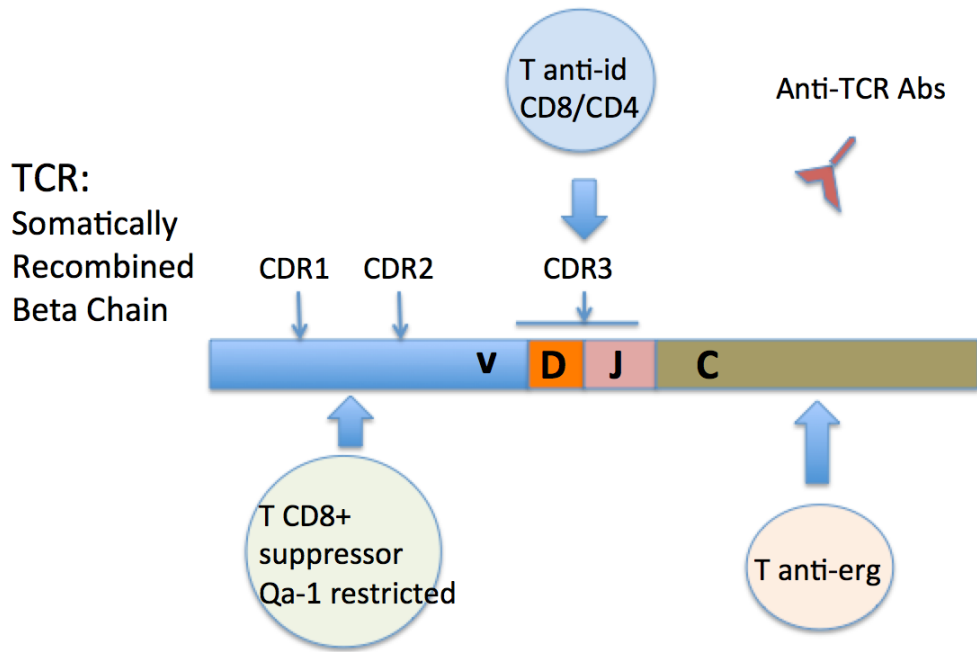


Figure 2. The beta chain of the TCR provides signals for regulation by anti-TCR antibodies and T cells: anti-CDR3 region (anti-id), anti-C region (anti-erg), and anti-V region (CD8 suppressors).

T anti-idiotypic (anti-id) regulation (Figures 1&2): T cell clones differ from one another by their somatically rearranged antigen receptor V (variable), D (diversity) and J (joining) mini-genes that encode its TCR and beta chains; TCR alpha chains do not include D segments [35, 36]. The CDR3 regions of the TCR, which interact with the antigen epitope presented in the MHC cleft, compose the T cell's idotype; so one might expect that a specific T cell vaccine should induce a regulatory immune response to its CDR3 idiotopes. Indeed, evidence that TCV induces anti-id T cell responses was obtained early on: TCV was found to activate T cells, both CD4 and CD8, that could distinguish between different T cell clones and their TCRs [37]; moreover, anti-id T cells induced by TCV could respond to peptides of TCR variable regions [38]; and such peptides were reported to be presented by activated T cells in association with MHC molecules [39]. Moreover, cytotoxic CD8 T cells induced by TCV could lyse autoimmune effector T cells [40], and TCV led to reduced numbers of specific autoimmune T cells in vaccinated subjects [41, 42].

Anti-id T cells alone, however, cannot explain all of the observations associated with effective TCV. First, TCV using a defined T cell clone or line responsive to a particular autoantigen is able to down-regulate autoimmune diseases known to target a number of different autoantigen epitopes on one or more self-antigens: for example, type 1 diabetes, known to involve effector autoimmunity to several different self-antigens, can be arrested by TCV with a single clone of T cells responsive to peptide p277, only one of the epitopes on HSP60, which is only one of the different target molecules [15]. Moreover, TCV may reduce, but not abolish T cell autoreactivity to the target self-antigens; although a vaccinated subject may resist the particular autoimmune disease, that subject can still maintain T cells reactive to the relevant self-antigens [43]. TCV affects the phenotype of the response to the antigen, not the ability of the subject's T cell repertoire to recognize that self-antigen [44]. In other words, TCV regulates the inflammatory nature of the autoimmune response, and not only the antigen recognition repertoire. An autoimmune disease reflects the pro-inflammatory phenotype of activated autoimmune T cells – the presence in the subject's repertoire of T cells that can recognize self-antigens is necessary, but not sufficient to cause a disease [45]. This implies that an autoimmune disease might be treated successfully by down-regulating the pro-inflammatory state of existing, activated T cells.

T anti-ergotypic (anti-erg) regulation (Figures 1&2): Shortly after the observation of anti-idiotypic regulation, we discovered that TCV also activated a T cell regulatory mechanism that was not directed to a

TCR idiotype, but to molecules expressed by activated T cells generally – a process we have termed anti-erg regulation [46]. In practice, rats could be rendered resistant to EAE by vaccinating them with T cells that did not respond to myelin antigens and that were not encephalitogenic. The critical requirement for this type of TCV was that the vaccinating T cells had to have been activated. The Greek word for *activation* or *work* is *ergon* (*ergon*) – hence we coined the terms *ergotope* (a molecule that marks a state of activation) and *anti-ergotypic* T cell (a T cell that responds to an ergotope and regulates an immune response). Functionally, we define an anti-erg T cell as any T cell that kills, proliferates or secretes cytokines when stimulated by syngeneic T cells that are in a state of activation; the anti-erg T cell does not respond to the same syngeneic T cells when they are not in an activated state. Anti-erg T cells may also be detected by their response to isolated ergotope antigens [47]. Anti-erg regulation focuses on the *state* of the system's T cells [48]; anti-id regulation, in contrast, focuses on the specificity of the system's T cells. The two targets of regulation would seem to complement each other.

We have observed (F. J. Quintana and I. R. Cohen; in preparation) that each of the two C region variants in the rat TCR includes ergotopes that are presented in the context of MHC II by rat T cells that have been activated by specific antigen – the same clones of T cells in a resting state do not present their C region ergotopes to anti-erg T cells. We found that a model autoimmune disease such as adjuvant arthritis [4] can be down-regulated significantly by the anti-C region anti-erg T cell regulatory response; inhibition of disease was associated with changes in the cytokine responses of the effector T cells to their target antigens. The protection against arthritis could be adoptively transferred using isolated anti-erg T cells. The details are being prepared for publication, but important for the present discussion is the fact that the C region of the TCR provides functional ergotopes, shown to be effective in down-regulating the inflammatory behavior of activated effector T cells. Note that the TCR C region is common to all effector T cells, irrespective of their specificity for self or foreign antigens; thus TCV appears to have uncovered a regulatory mechanism that operates generally to down-regulate inflammation induced by any T cell immune response.

Anti-erg T cells are heterogeneous; they are present in the thymuses of newborn rats and are found in the spleen some days later [49]. They include ab TCR CD4 and CD8 T cells restricted respectively by MHC II and MHC I molecules expressed on the stimulating, activated T cell or on other types of APC. Anti-erg T cells also include gd TCR T cells that may not be MHC restricted [50]. Some anti-erg T cells proliferate in response to ergotopes without secreting detectable cytokines; some secrete different sets of regulatory cytokines including IL-10, IL-4, IL-2, TGFb, TNFa, and IFNg [51]. Anti-erg T cells were first demonstrated following TCV, but they are detectable in naïve humans and rodents; they expand in response to strong immunization to foreign antigens such as complete Freund's adjuvant [51]; they appear to be decreased in subjects suffering from MS, but expand after TCV [51].

CD-25 ergotope: In addition to the TCR C region, other peptide ergotopes are presented by activated T cells, including peptides of the CD-25 molecular component of the alpha chain of the IL-2 receptor (Figure 1), which is up-regulated upon T cell activation [44, 52]. The CD-132 g chain component of the IL-2 receptor, which is not up-regulated in activated T cells, does not feature ergotope peptides. The TNF receptor also provides ergotopes presented by activated T cells [53].

HSP60 ergotopes: A notable source of ergotope peptides is the HSP60 molecule (Figure 1); activated T cells present HSP60 peptide epitopes in the clasp of their MHC II molecules, which are also up-regulated, to anti-erg regulatory T cells [47]. HSP60 and other stress protein molecules function within the cell as chaperones to assist in the proper folding of newly synthesized proteins and to protect the cell from denatured proteins, among other roles [47, 54]. The essential functions of HSP molecules for the health of cells and organisms make them ideal signals for the immune system – the expression of HSP molecules reflects the states of cells and tissues; these molecules provide the immune system with reliable biomarkers, which assist the immune system in regulating inflammation [55]. The HSP60 molecule is central to a network of immune cells, in which its molecular concentration, its particular peptide moiety, and the cell types that respond to it are integrated to determine a pro-inflammatory or anti-inflammatory outcome [54].

Moreover, we have discovered that HSP60 is immunologically related to HSP70 and HSP90 as immune system regulatory signals: intramuscular administration of DNA encoding HSP70 or HSP90, like administration of DNA encoding HSP60 [56, 57], inhibits autoimmune disease [58]. We found that the three HSP molecules are mutually connected in a network – administration of HSP70 or HSP90 induces regulatory T cells that respond to epitopes of HSP60 as well as regulatory T cells that respond to HSP70 and HSP90 epitopes [58].

A peptide derived from human HSP60 – p277 – is a T cell ergotope recognized by anti-erg regulatory T cells [47]. This HSP60 ergotope peptide [59] is presently completing a phase III clinical trial to arrest the autoimmune destruction of Beta Cells in type 1 diabetes. In addition to serving as a T cell ergotope, peptide p277 is an agonist by way of Toll-like Receptor 2 (TLR2) signaling in CD4+CD25+ Tregs [60]. Below, we shall note that HSP60 epitopes are also presented by the Qa1 antigen-presenting molecule to CD8+ suppressor T cells; HSP60 is truly a multi-potent, key regulator of inflammation.

CD4+Tregs: Classical Tregs of the CD4+ type [61], now defined by various markers – CD4+CD25+FoxP3, etc – had not yet been discovered when TCV first came to the attention of immunologists, so work on CD4+ Tregs in the context of TCV was late and has been scanty. However, there has been at least one report of standard Tregs induced by TCV [44]. In a clinical trial of TCV in rheumatoid arthritis (RA) patients, Jingwu Zhang and associates constructed T cell vaccines using autologous synovial fluid T cells cultured in low concentrations of IL-2 to preferentially expand T cells that had already been activated in the synovial exudate in vivo. They reported clinical and immunologic improvement in two-thirds of the treated subjects associated with CD8+ cytotoxic T cells specific for the T cell vaccine along with CD4+CD25+Foxp3hi, IL-10 secreting Tregs reactive to peptides of CD-25, the IL-2 receptor α -chain [44]. It is likely that the vaccine-specific CD8+ cytotoxic T cells were anti-id and that the Tregs were anti-erg, as we have defined them here. Thus, human subjects respond to TCV with a combination of anti-id and anti-erg regulators that include classical CD4+ Tregs.

Qa1-restricted CD8+ suppressor T cells (Figures 1&2): CD8+ suppressor T cells were first discovered in the 1970s by Gershon and colleagues [62], but molecular-scale investigation of this class of cells was delayed for decades until the emergence of new molecular technologies made it possible to revisit the subject [63]. Even now, however, the relatively newer CD4+ Tregs have gained most of the attention; but, irrespective of research fashions, these CD8+ regulatory cells are relevant to TCV. The important points for our present discussion are that 1) these suppressors are targeted to epitopes presented by the non-classical Qa-1 antigen-presenting molecule (so named in mice; the human equivalent is termed HLA-E) [64]; 2) Qa-1 restricted epitopes include peptides of the V regions of the TCR [65] and peptides of the HSP60 molecule [63]; 3) a range of epitopes is presented by Qa-1 depending on the state of the presenting cell – activated T cells, but not resting T cells present their V region peptides [66, 67]; 4) Qa-1-restricted epitopes can be cross-presented by non-T cell APC to the CD8+ T regulators [63-67]; and, 5) most importantly for us here, these CD8+ regulators are activated by TCV and contribute significantly to TCV-induced regulation of inflammation [63-67].

Anti-TCR antibodies (Figures 1&2): TCV has been reported to induce antibodies with regulatory properties [34, 68]. Much remains to be learned about these antibodies, their target epitopes on the TCR and their regulatory functions, but we can list several of their relevant features: 1) anti-TCR binding antibodies can be detected following TCV, but also following spontaneous recovery from an autoimmune disease and following intense immunization to a foreign antigen [34]; 2) anti-TCR antibodies may be anti-id [69], but TCV can also induce antibodies to many other epitopes expressed by activated T cells [33]; 3) post-TCV antibodies bind to activated T cells much more than they do to resting T cells [34] – the antibodies, like TCV-induced anti-erg T cells, recognize the activated state of the T cell they regulate; 4) post-TCV antibodies can down-regulate T cell responses to self or foreign antigens [34, 68]; and 5) anti-T cell antibodies stimulated by a foreign T cell vaccine can down-regulate an autoimmune disease response [34] – these antibodies too are anti-erg. Thus we can conclude that regulatory antibodies, and not only regulatory T cells are functionally activated by TCV. We have yet to learn how these antibodies down-regulate inflammation, but it is possible that anti TCR-antibodies induced by TCV trigger T cell activation in a way that results in T cell deletion and/or anergy.

Public CDR3 domains and immune regulation

The recent discovery of shared, public CDR3 domains of the TCR [70] casts a new light on TCV anti-id regulation. Research on public CDR3 domains is in an early stage, and we cannot yet draw firm conclusions; nevertheless, the findings invite us to consider the possible roles of public TCR segments in antigen-specific T cell regulation.

What is a public CDR3 domain? The CDR3 segment of the TCR is formed by recombination of V-D-J mini-gene segments (Figure 2); this recombination event has been assumed to be diversely random, and the diversity of the CDR3 segment is further amplified by insertions and deletions of DNA nucleotide sequences not encoded in the genome. Diversification of the TCR is thought to fashion about 10 billion possible CDR3 amino acid sequences [35, 36]; this is larger than the estimated number of T cell specificities in an individual mouse or human (10^7 - 10^9). Thus, on statistical grounds, the expression of identical CDR3 sequences in different subjects would be unexpected.

Despite expectations, we have recently reported that the naïve mouse T cell repertoire contains CDR3 TCR beta chain amino acid sequences shared by most mice; such highly shared sequences can be termed *public*. We sequenced hundreds of thousands of TCR molecules present in the CD4⁺ T cells obtained from the spleens of 28 young and healthy C57BL/6 mice; about three hundred public CDR3 sequences were found to be expressed in all 28 mice, and over a thousand sequences were shared by 70% or more of the mice [70].

These highly shared CDR3 sequences differed in two features from the more private sequences found in only one or a few mice: the more private sequences exhibited a range of frequencies from very low (small clone size) to very high (large clone size); the more public sequences, in contrast, were all found at relatively high frequency – low frequency sequences were missing. Since a high frequency T cell is assumed to have been expanded in response to antigen stimulation [70], we can surmise that all the T cells bearing public CDR3 sequences had undergone positive selection, probably in response to antigen stimulation; in contrast, only some T cells among the more private sequences were likely to have been expanded by contact with their specific antigens.

Another feature of public CDR3 sequences was their high degree of *convergent recombination* [70]. Due to codon degeneracy, a single CDR3 amino acid sequence can be encoded by more than one nucleic acid recombination event. We found that private CDR3 amino acid sequences were derived from an average of only one nucleic acid recombination event; public sequences, in contrast, were each encoded by many different nucleic acid recombinations – the average number of nucleic acid recombinations that converged to encode a single public amino acid sequence was 35; some individual public amino acid CDR3 sequences were the products of up to a hundred different nucleic acid recombinations in the mouse population. A high degree of convergent recombination could be most easily explained by the positive selection of different nucleic acid recombinations all encoding the same amino acid sequence. What can explain this selective expansion?

It has been proposed that public TCR sequences could emerge from biases in the process of V-D-J recombination [71, 72]. Indeed, the results of our computer simulations suggested that there does exist a fundamental biochemical-mechanical bias in particular V-D-J nucleic acid recombination partners, probably due to favorable positioning of certain V, D and J genes on the chromosome [70]. Our experimental observations suggested that recombination bias, as estimated from TCR repertoire data, can explain the higher frequencies of many public CDR3 sequences, as well as the high level of convergent recombination for these sequences (70). This biophysical bias in particular V-D-J nucleic acid recombination partners would appear to be further fine-tuned by antigen selection. Public sequences thus result both from recombination bias and from subsequent antigen selection in different individuals [70].

Obviously, the T cell repertoires of different subjects cannot be coordinated at the somatic level – my T cells can know nothing about the specificities of your T cells. Although the TCR is created by somatic V-D-J recombination in each individual, CDR3 *publicness* must be a product of the evolutionary experience of the species encoded in germline DNA inherited in common by different individuals. Thus the germline would have to encode both favorable V-D-J positioning on the chromosome and TCR selection by certain self-antigens common to different individuals. In contrast to public CDR3 sequences, private sequences would entail somatic selection restricted to the level of the individual. This hypothesis would gain credibility if indeed public and private CDR3 sequences would be found to recognize different classes of antigens. How can one derive a likely target antigen from a CDR3 TCR sequence?

At the present state of immunology, knowledge of a CDR3 amino acid sequence alone cannot tell us the sequence of the antigen epitope recognized by that TCR; this uncertainty is compounded by the fact that the functioning TCR is formed by the association of both alpha and beta chains. So the sequences of CDR3 beta chains, public or private, cannot tell us which antigens the TCR sequences might recognize. However,

we were able to circumvent this limitation to some degree and annotate our CDR3 sequences by searching for identical sequences present in T cell clones of known specificities published in the past.

Over the years, various laboratories have published the TCR sequences of T cells associated with variously specific immune reactivities; any of our CDR3 TCR β sequences identical to any of these annotated TCR β sequences would suggest that our naïve C57BL/6 sequences might be associated with a similar immune function. We culled from the literature some 250 TCR beta chain sequences obtained from various strains of mice (with different MHC haplotypes) associated with known immune reactivities; amazingly, 124 of the 250 annotated CDR3 TCR beta chain sequences were present in our CDR3 database.

The use of identical CDR3 sequences by mice of differing MHC genotypes can be explained by the fact that the CDR1 and CDR2 regions of the TCR, which bind to the antigen-presenting MHC molecules [73], are present in the V region and not in the V-D-J joining region (Figure 2); thus MHC restrictions are more linked to the V region than they are to the CDR3 joining region. Different MHC molecules that can present the same peptide epitope in their clasp could select a common CDR3 region appended to different TCR V segments [70]. Indeed, we find that the same CDR3 region can be encoded by a number of different V genes, and the V β genes used to enclode a given CDR3 sequence vary between mice of different MHC haplotype (70).

We sorted the 124 public CDR3 sequences into four annotated T cell categories: TCR responses to foreign antigens associated with pathogens; TCR responses to known self-antigens or associated with autoimmune conditions; TCRs from T cells infiltrating tumors or responding to known tumor antigens; and TCRs associated with allograft reactions. Most tumor antigens are self-antigens or modified self-antigens, and allograft responses include anti-self reactions [74, 75]. Thus, the four categories of annotated T cell reactivity could be reduced to two: anti-foreign and anti-self associations. These two categories nicely separated the private and public CDR3 sequences: the more private CDR3 sequences were prominent in the anti-foreign category and deficient in the anti-self category; the more public CDR3 sequences were present in the anti-foreign category, but they were relatively enriched in the anti-self category [70].

Note that publicness constitutes an unanticipated distinction between self and not-self antigens: both types of antigen are recognized by the T cell repertoire, but the TCRs for self tend to be more public and those for foreign tend to be more private. This distinction, of course, is quantitatively relative, not absolute.

The association of a set of public CDR3 segments with self and self-like reactivity in healthy mice is compatible with the Immunological Homunculus theory – autoimmune repertoires of T cells and B cells in healthy individuals serve an ongoing dialog between the immune system and the body [76, 77]. Be that as it may, does the set of public CDR3 sequences serve TCV and immune system regulation? The story of the C9 T cell clone suggests a positive answer, at least for one member of the public set.

C9 CDR3 regulatory functions: We isolated the C9 T cell clone in 1991 from NOD strain mice on their way to spontaneously develop type 1 diabetes (T1D) [78] and reported its TCR alpha and beta chain sequence in 1999 [79]. The C9 clone responded to human or mouse HSP60 and to a defined peptide of the human HSP60 molecule, peptide p277 [78, 80]. Clone C9 was able to adoptively transfer insulinitis and hyperglycemia to otherwise healthy recipient mice; albeit, the transferred diabetes was mild and transient [78]; after spontaneous recovery, the mice were no longer susceptible to T1D. The functional relevance of clone C9 to autoimmune regulation was further confirmed by its ability to vaccinate NOD mice against the spontaneous development of autoimmune type T1D [78]. T1D is known to be associated with autoimmunity to numerous self-antigens [81] including insulin and glutamic acid decarboxylase; despite the multiple autoimmune targets, C9 was an effective T cell vaccine.

We found that the CDR3 beta chain peptide segment of the C9 TCR was public in different NOD mice [79], and the peptide itself could be used to vaccinate against T1D [78, 82]. The outcome of this TCV-induced inhibition of T1D appeared to involve a cytokine shift to an anti-inflammatory phenotype in the diabetogenic T cells, rather than to their elimination [83].

Interestingly, inhibition of T1D in NOD mice could be effected by a single subcutaneous administration of 100 micrograms of the target peptide of C9, p277 [82]. The regulatory effects of p277 appear to be relevant to humans too; administration several times a year of 1 mg of p277 subcutaneously preserved beta-cell function in humans with newly diagnosed type 1 diabetes [59, 84], and p277 peptide therapy is presently completing a phase 3 clinical trial. The half-life of peptide p277 in human plasma or

serum is about 3 minutes; hence, resistance to T1D is mediated by an endogenous regulatory mechanism set into motion by a transient encounter with the peptide.

Indeed, down-regulation of T1D by peptide p277 in NOD mice is marked by the activation of anti-C9 anti-id T cells specific for the CDR3 peptide of C9 [15]; likewise, vaccination with the C9 CDR3 peptide up-regulates the anti-C9 anti-id response and down-regulates the inflammatory phenotype of the spontaneous T cell response to p277 [15]. Moreover, adoptive transfer of anti-C9 anti-id T cells also inhibits T1D [15]. Thus an immune regulatory network links the public C9 CDR3 domain of the TCR repertoire with anti-id T cells and with the p277 peptide of HSP60. Figure 3 shows that the anti-C9 anti-id is the agent that down-regulates the autoimmune T1D, and this anti-id is activated by the public CDR3 segment of the C9 TCR. Thus, T1D can be down-regulated alternatively by vaccinating with C9, by administering its p277 target self-antigen, or by adoptively transferring anti-C9 anti-id T cells. In other words, C9 vaccination activates anti-C9 anti-id down-regulators of inflammation; peptide p277, via presentation by APC, activates C9 to activate, in turn, anti-C9 anti-id down-regulators.

Autoimmune & Inflammatory Disease Inhibition

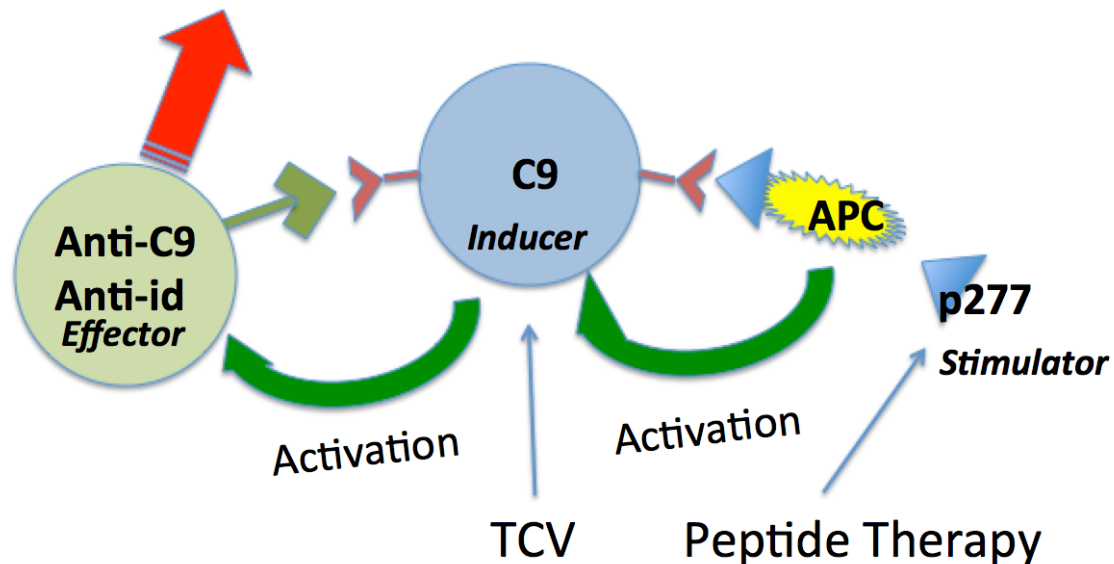


Figure 3. Autoimmune and inflammatory disease inhibition. The C9 T cell illustrates regulatory network connectivity: the effector of regulation is the anti-C9 anti-id response; C9 is the inducer of this effector of regulation; and peptide p277, presented to C9 by APC, is a stimulator of the C9 inducer; TCV using C9 T cells directly induces the anti-C9 regulatory response. C9 thus connects peptide therapy with TCV.

This sounds dauntingly complicated, but we might simplify C9-based regulation thusly: take another look at Figure 3; the actual *effector of regulation* is the anti-C9 response; C9 is the *inducer of regulation*; and peptide p277 is the *stimulator* of the inducer. Peptide p277 is also a co-activator of CD4⁺ Tregs via Toll-like Receptor 4 (TLR4) of the Tregs [60]. The CDR3 idiotope of the C9 inducer is at the center of this regulatory network. Do other public CDR3 idiotopes play similar roles as inducers of regulation? If so, anti-id responses directed to public CDR3 segments might be exploited generically to facilitate the enforcement of immune regulation. Indeed, public CDR3 segments might provide anti-id regulation with an anti-erg character widely shared among different individuals. Obviously, these speculations are only working hypotheses. The fact that naïve, healthy C57BL/6 mice harbor public TCR sequences such as C9 [70], which are also expressed in the autoimmune T1D process in NOD mice, suggests that such regulatory networks are built into the physiology of the healthy immune system [76, 77].

Regulation depends on tissue context

Most autoimmune diseases result from the activities of a large number of activated, autoimmune T cells – no autoimmune T cells, no autoimmune disease. How then does TCV down-regulate an inflammatory autoimmune disease if it merely adds to the body more activated, autoimmune T cells? Obviously, the TCV cells are attenuated, so they are harmless – but even so, how does TCV down-regulate the disease process? What does the vaccine do to the host immune system that the endogenous pathogenic T cells don't do? This question brings us to the regulation of the regulators.

The regulators of activated T cells induced by TCV down-regulate the effectors, but the regulators too need to be down-regulated. The reason is obvious: unless these regulators get removed, the immune system would become paralyzed by regulator-cell overload; after all, immunity to pathogens and tumor surveillance requires vigorous effector responses. There are probably a number of different mechanisms that work to restrain over-active immune suppression. We have uncovered one way that helps keep regulators and effectors balanced:

Figure 4 illustrates our finding that anti-erg regulators are subject to loss or renewal, depending on who presents them with their target ergotope: anti-erg regulators become inactivated following a direct interaction with effector T cells presenting ergotopes, but the anti-erg regulators proliferate when they interact with APC (non-T cell) that present the same ergotope antigens [47, 85]. Thus the cellular context can direct the outcome; at sites of inflammation, the anti-erg T cells are lost when they down-regulate activated effector T cells – the effectors are inactivated, but so are the regulators. But at non-inflamed sites outside the diseased tissue, the regulators proliferate when they interact with APC (non-T cell) that cross-present T cell ergotopes. Inflammatory sites are marked by concentrations of cytokines and other innate receptor agonists that are absent at non-inflamed sites. Outside a site of inflammation, the ergotope protein, its peptide ergotope or even DNA encoding the regulatory ergotope can all induce expansion of the regulators when mediated by APC (non-T cell) (Quintana, FJ and Cohen IR; in preparation).

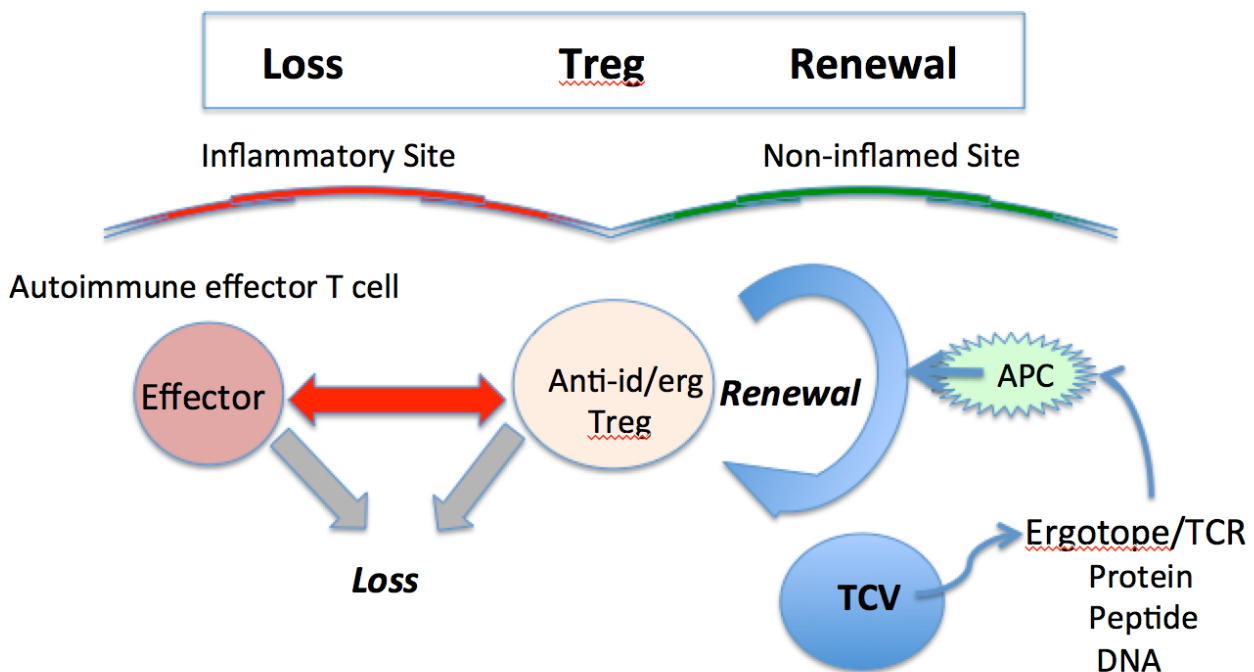


Figure 4. The regulation of the regulators. Tregs (anti-id/erg) undergo loss or renewal depending on the context of inflammation and the type of APC that presents the regulatory simulator signal. At the site of inflammation, the Tregs are lost when they down-regulate the effector T cells; but the same Tregs can be stimulated to proliferate and renew regulation when they encounter their target id or erg presented by non-T cell APC at non-inflamed sites. The target id or erg can be presented directly via TCV or cross-presented by APC that have taken up the relevant id/erg protein, peptide or DNA encoding the protein.

Likewise, the anatomic context of T cell activation would also seem to be important for generating autoimmune effector T cells; it has been proposed that the autoimmune effectors of MS are generated outside the nervous system, possibly in the gut [86].

In any case, one might reason that TCV using attenuated effector cells, or even a small number of intact effector cells administered into a peaceful body site, such as the subcutaneous tissue, enables APC cross-presentation of T cell ergotopes and idiotopes or even of T cell DNA that leads to the activation and expanded renewal of anti-erg and anti-id T regulators, free of interference by inflammatory effector T cells. Indeed, we have found that naked DNA encoding ergotopes such as CD-25 [47], HSP60/70/90 [56-58], and the TCR C region (in preparation) can each induce down-regulation of model autoimmune diseases. Once stimulated by APC presenting the ergotope, the amplified regulators then can migrate to the site of inflammation where they down-regulate the effectors, while also getting themselves down-regulated in the process of regulation. Note that the generation of CD8⁺ T suppressors has been shown to be mediated by APC (non-T cell) that present the TCR idiotope to the regulatory T cells [67]. Thus, the exhaustion or renewal of regulators depends on the tissue site and cellular context of the reaction.

Epilogue

TCV can teach a number of key points about immune regulation: First, the immune system is capable of regulating its own inflammatory reactivity; one can reason, in fact, that endogenous self-regulation is going on all the time [76, 77]. The relapsing-remitting phase of MS is probably a clinical expression of the dynamic struggle between effectors and regulators. TCV can be seen in this light as a way to enhance the collective strength of the regulators. The safety and effectiveness of TCV can be attributed to its activation of endogenous agents of regulation; TCV talks to the system (Figure 3), and does not punish it with pharmaceutical suppression.

Secondly, the TCR and other molecules involved in T cell activation are prime targets for regulators (Figures 1 and 2). Regulation is expressed by networks (Figure 3).

Thirdly, TCV shows us that regulation is mediated by variously different populations of T cells and antibodies directed to diverse biomarker molecules; regulation is not the exclusive domain of CD4⁺ Tregs (Figure 1). Regulation can be antigen-specific (anti-id T cells and antibodies) or antigen-non-specific (anti-erg responses). Regulation is an expression of systems immunology.

Fourthly, some mechanisms of regulation are sensitive to the contexts of inflammation and the anatomy of interactions of different cell types (Figure 4).

Fifthly, regulation is structured by evolutionary experience that has generated regulatory repertoires shared by different individuals – manifested as an Immunological Homunculus [87] and public CDR3 sequences [70].

Perhaps the time is ripe for individualized, cell-based, physiological treatments.

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