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Adaptive T cell tuning in immune regulation and immunotherapy of autoimmune diseases

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Invited Review

Adaptive T cell tuning in immune regulation and immunotherapy of autoimmune diseases[☆]

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ABSTRACT

Lymphocyte receptors confer antigen specificity on the adaptive immune response. Increasing evidence points to the role of adaptive tuning particularly amongst CD4^{+} T cell responses. This review summarises how T cell tuning impacts on critically important aspects of immune regulation including thymic selection, the immune response to chronic antigen exposure and antigen-specific immunotherapy of autoimmune conditions. Recent work has revealed a novel mechanism for T cell anergy and regulatory type 1 T cell differentiation through a limitation of T cell receptor mediated signalling combined with epigenetic priming of tolerance associated genes.

1. Introduction

Much of the work of our laboratory has concerned mechanisms of fine tuning of the adaptive immune response largely focusing on the activity of autoreactive CD4 T cells. We are indebted to Zvi Grossman and Bill Paul who in 1992 published a theoretical paper on adaptive cellular interactions in the immune system [1]. Their tunable activation threshold model correlated with many of the observations arising from our laboratory at that time and has since formed a theoretical framework underpinning much of our work. Their view was that "Dynamic tuning of cellular responsiveness, as a result of repeated stimuli, improves the ability of cells to distinguish physiologically meaningful signals from each other and from noise. In particular, lymphocyte activation thresholds are subject to tuning, which contributes to maintaining tolerance to self-antigens and persisting foreign antigens, averting autoimmunity and immune pathogenesis" [2]. Since their original paper was published, there has been increasing evidence that background or 'tonic' T cell receptor (TCR) signalling tunes the immune response to antigen. A moderate level of tonic signalling is required for an optimal T cell response [3] whereas stronger tonic signalling, as evidenced by activation marker expression, leads to a reduced response or desensitisation.

Our laboratory has studied a model self-antigen, the N-terminal antigenic epitope of myelin basic protein (Ac1–9 AcASQKRPSQR) capable of inducing autoreactive CD4 T cells and experimental autoimmune encephalomyelitis (EAE) in H-2[u] mice when injected with strong adjuvant [4]. Early work identified the amino acids of this epitope responsible for determining either TCR or MHC class II interactions [5]. Subsequent work showed that the amino acid at position four of Ac1–9 determined interaction with class II MHC H-2 A[u]. Importantly, lysine in the native peptide led to weak interaction whereas increasingly hydrophobic amino acids produced much stronger binding (Table 1) [6]. This knowledge allowed us to generate a panel of peptides (4Y>>4A>4K) that change the strength of signal in T cells in vitro and in vivo. In addition to studies in conventional H-2[u] mice much of our work utilised the Tg4 mouse expressing a TCR specific for Ac1–9 [7].

2. Signal strength and thymic selection

It is well accepted that thymic selection of CD4 T cells depends on strength of signal [8]. Nascent CD4 cells are required to have weak affinity for one of the MHC class II molecules expressed in the thymus to be positively selected and survive (Fig. 1). On the other hand, they are deleted if they interact strongly with a self-antigen-MHC complex in the thymus. For example, injection of peptide analogues of increasing affinity for MHC (4Y>>4A>4K) led to affinity dependant deletion of developing thymocytes in the Tg4 mouse [7].

The majority of cells generating a nascent TCR in the thymus fail to find an MHC-peptide complex that they interact with well enough to survive and are lost by neglect. Furthermore, cells binding with an

Table 1
This data including incidence of experimental autoimmune encephalomyelitis (EAE) in H-2^u mice is summarised from ref 11.

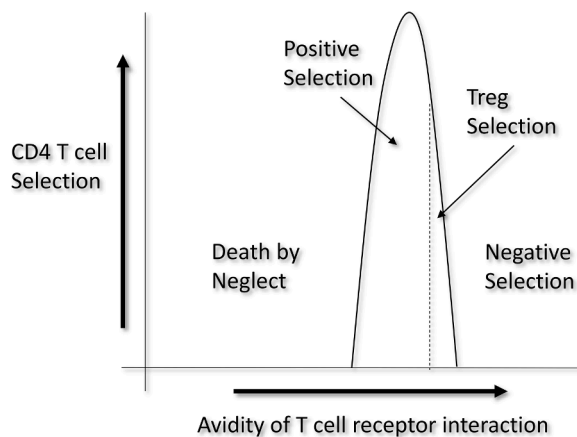| Peptide Sequence	Abbreviation	Relative binding affinity for A^u	Incidence of EAE (%)
AcASQKRPSQR	4K	<0.00001	60
AcASQARPSQR	4A	0.01	10
AcASQVRPSQR	4V	0.2	0
AcASQYRPSQR	4Y	1	0

Fig. 1. Selection of CD4 T cells in the thymus depends on the avidity of T cell receptor interaction with MHC class II molecules and their associated peptides. Avidity of T cell receptor recognition translates to strength of signal and this has a direct consequence on T cell selection [8]. The level of signal strength must reach a threshold for nascent T cells to survive and hence undergo positive selection. Presentation of antigen by medullary antigen presenting cells including dendritic cells and thymic epithelial cells expressing the AIRE gene, leads to deletion, or negative selection, of those CD4 cells recognising self MHC II directly or self-antigens presented by MHC II [43]. There is evidence that cells with a high but intermediate avidity are selected as Foxp3$^+$ regulatory T cells and that this depends on the balance of signalling through T cell receptor and the costimulatory molecule CD28 [10].

avidity at the higher end of those CD4$^+$ cells that are positively selected may upregulate Foxp3 and exit the thymus as regulatory T (Treg) cells (Fig. 1). This is governed by the combined strength of signal provided by TCR and CD28 signalling with the latter counterbalanced by CTLA4. We were able to show that the avidity of T cells selected in the Tg4 mouse depends on expression of CTLA4 [9, 10]. CTLA4 deficient Tg4 mice select a repertoire of lower affinity TCRs because they receive a higher level of CD28 signalling. These CTLA-4 deficient mice generate a high proportion of Foxp3$^+$ cells in a CD28 dependant fashion. We were able to prove that T cells expressing a TCR normally found in potentially pathogenic T cells were selected as Foxp3$^+$ Treg cells in CTLA4-deficient mice.

3. The distinction between antigenicity and immunogenicity

The strength of signal resulting from the stability of an MHC-peptide complex determines levels of CD4 T cell activation, IL-2 production and proliferation *in vitro*. The 4Y>>4A>4K panel of peptides showed a direct correlation between MHC binding and antigenicity with T cells responding to >1000 fold lower dose of the 4Y analogue when compared with the wild-type 4K peptide [6]. However, this correlation between MHC-binding affinity and antigenicity does not apply to the immunogenicity of the antigens. Injection of the high affinity 4A analogue of Ac1–9 in complete Freund's adjuvant suppressed the response to the wild-type 4K peptide and prevented induction of EAE

[5]. Subsequent studies showed that the higher MHC-binding affinity of the 4Y analogue caused depletion of 4K-reactive CD4 cells with high affinity TCRs through Fas-mediated activation induced cell death (Fig. 2) [11]. Therefore, increasing the strength of signal delivered via the TCR, in combination with strong costimulation induced by adjuvant, results in tuning of the 4K-reactive T cell repertoire through deletion of cells with high affinity TCRs [11]. An analysis of the dose of peptide required for optimal recall of lymph node cells from non-transgenic H-2^u mice immunised with the 4Y>>4A>4K panel of peptides revealed evidence of signal strength dependent tuning *in vivo*. B10.PL mice were immunised with each individual peptide in complete Freund's adjuvant and the resulting primed lymphocytes restimulated *in vitro*. Cells from the primed mice responded to the homologous peptide at the same dose *in vitro* (10 μM) demonstrating that the immune response had been tuned to this optimal recall dose of antigen depending on the affinity of the peptide for MHC and the resulting strength of signal [11].

4. Response to chronic antigen exposure

As discussed above it is clear that there is an intrinsic tuning mechanism for the T cell repertoire involving Fas-mediated activation induced cell death. Studies in humans and mice have revealed an additional mechanism by which the immune repertoire accommodates chronic infection and self-regulates [12]. Mice chronically infected with pathogens such as Toxoplasma gondii and Leishmania major suppress the immune pathology that would arise from an overaggressive Th1 response to the pathogen by generation of Foxp3$^-$, IL-10 secreting Tr1 cells [13, 14]. These cells are anergic, T-bet expressing cells derived from antigen-specific Th1 cells which through chronic exposure to antigen convert from a potentially pathogenic to a regulatory phenotype, reduce inflammation in infected individuals and thereby save the host from death due to excessive immune pathology.

Repeated exposure to antigen has been used for more than 100 years as a treatment for allergy [15]. The mechanism of desensitization with allergen appears to involve a switch from a Th2 to a Tr1 cell response to the allergen analogous to that seen in chronically infected individuals. This was elegantly demonstrated in beekeepers where chronic exposure to allergen through repeated bee stings resulted in a shift in T cell response from IL-4, IL-5 and IL-13 producing Th2 to IL-10 secreting Tr1 cells [16]. Similar observations were made in both humans and mouse models in response to allergen derived CD4 T cell epitopes [17, 18]. Others have suggested that the mechanism of allergen desensitization involves the generation of an IL-10 dominated T cell response associated with a shift from IgE to allergen blocking IgG4 antibodies [19]. Importantly, Altin and colleagues have shown that chronic exposure to a Th2-associated antigen generates a population of GATA-3$^+$, IL-10 producing cells that are Foxp3$^-$ and CTLA-4$^+$. These cells suppress proliferation of naïve CD4$^+$ cells and are derived from conventional Th2 cells [20]. The accumulating evidence points to the generation of IL-10 producing T cells that can derive from conventional Th1 (Tbet$^+$), Th2 (GATA-3$^+$) or Th17 (RORγt$^+$) cells as a natural, negative-feedback mechanism designed to limit the immune pathology caused by chronic infection and exposure to antigen [21].

5. Development of antigen-specific immunotherapy for autoimmune diseases

Current treatments for autoimmune diseases aim to treat the symptoms rather than suppressing the underlying pathology. Furthermore, many of the current treatments for autoimmune diseases utilise non-specific immunosuppressive agents that heighten the risk of cancer and infectious diseases. This latter point is particularly pertinent at the current time where patients treated with strong immunosuppressive drugs fail to respond well to vaccination and can suffer worse consequences of SARS-CoV-2 infection. There is a clear need to develop treatments that focus on the specific cells driving autoimmune diseases.
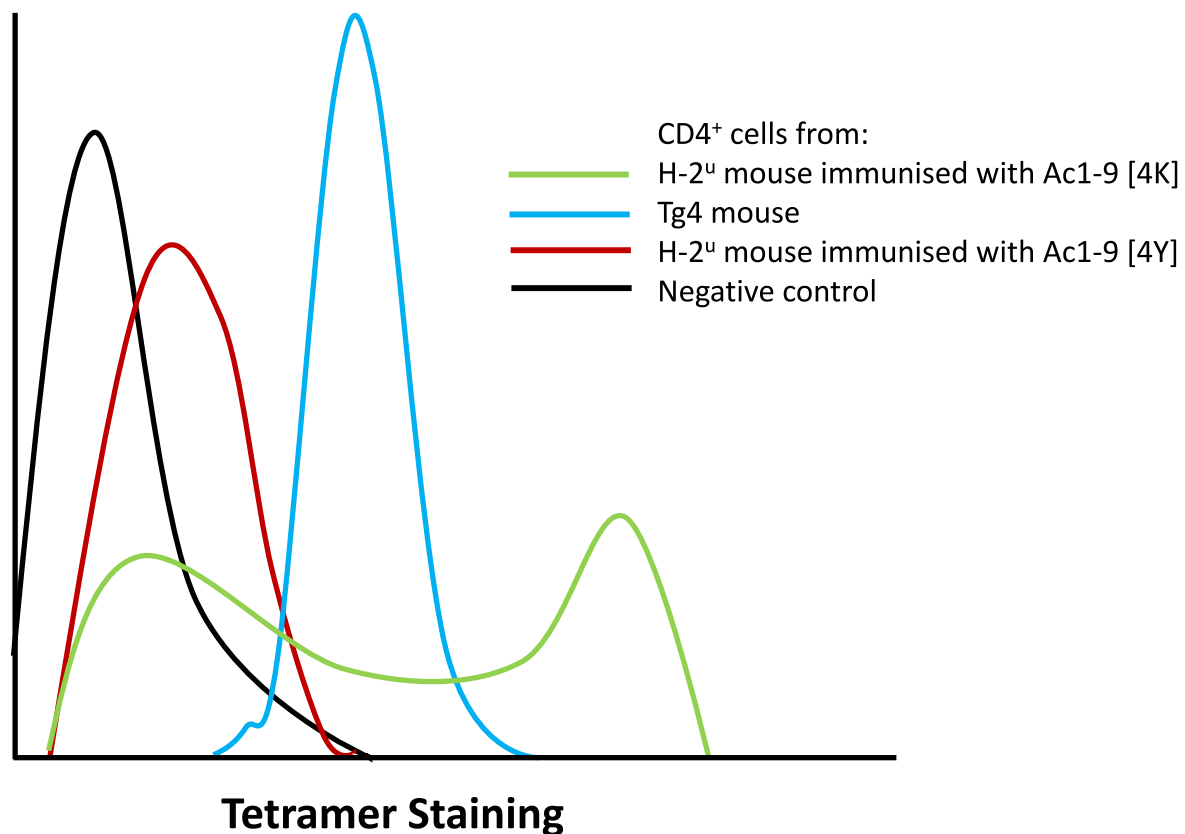
Tetramer Staining

Fig. 2. This figure represents how altering the strength of signal results in adaptive tuning of the T cell repertoire and is based on data published by Anderton et al [11]. Cell lines were prepared from A^u mice either transgenic for the Tg4 TCR specific for MBP Ac1–9 or alternatively from mice immunised with the low affinity [4 K] or high affinity MHC binding [4Y] analogues of MBP Ac1–9. Cell lines were stained with tetramers prepared from A^u-4Y monomers [44]. While the Tg4 cell line contains a homogeneously staining population, the cell line from 4 K immunised mice has TCRs ranging from low to high affinity. Mice immunised with the high affinity 4Y analogue, on the other hand, only generate cells with relatively low affinity TCRs. The relative affinity of the cell lines for the MHC-4Y tetramer was confirmed by elution experiments where the off rate followed the order 4Y>>Tg4>4 K showing that immunisation with the high affinity 4Y analogue resulted in deletion of cells with high affinity TCRs [11].

We believe that this is now possible thanks to the development of antigen-specific immunotherapy based on our increasing knowledge of the nature of autoantigens [22]. However, unlike with allergens, use of intact antigen has not been successful and has led to induction of pathogenic autoantibodies in models of MS and Graves' disease [23, 24] or cytotoxic T cells in the case of type I diabetes [25]. We have argued that it should be sufficient to selectively and specifically inhibit antigen-specific CD4 T cells to treat autoimmune diseases [26]. We propose that this can be achieved by administration of soluble peptides designed as antigen processing independent T-cell epitopes or apitopes. Arguably, the greatest breakthrough in the development of antigen-specific immunotherapy with T-cell epitopes came when we demonstrated that soluble peptide epitopes administered by various routes [27–29] would induce tolerance whereas oral epitope or intact antigen was ineffective in mouse models of MS [27, 30]. Our early work showed that the Ac1–9 N-terminal peptide of MBP behaves as an apitope and that administration of the 4Y analogue of Ac1–9 induces anergy through suppression of IL-2 production and a shift towards IL-10 production [31]. The resulting Tr1 cells were shown to be capable of antigen induced suppression *in vitro* and, more importantly, IL-10 dependant suppression of bystander T cell activation *in vivo* [31]. In the past decade, our group has defined the properties of apitopes and revealed their mode of action *in vivo*. The peptides must be soluble allowing them to traffic to lymphoid organs following injection. Importantly, the peptides are shown to bind directly to MHC class II molecules on the surface of steady-state dendritic cells [32]. Steady-state dendritic cells have peptide receptive MHC class II proteins at the cell surface because they do not acidify antigen processing compartments within the cell as effectively as mature dendritic cells, B cells and macrophages [33]. Furthermore, steady-state dendritic cells express low levels of costimulatory molecules, CD80 and CD86 and hence induce anergy. Burton and colleagues have defined changes in gene expression that result from repeated administration of soluble apitopes in the Tg4 mouse model. Tg4 mice bred onto the RAG gene-deficient background develop spontaneous EAE at approximately 11 weeks of age. Six doses of the model 4Y apitope given between six and eight weeks of age were sufficient to protect the mice from disease for life. Burton et al. showed that the degree of anergy, IL-10 production and suppression *in vitro* and *in vivo* were strictly dose-dependant. This study showed that repeated administration of apitope peptides induces a sequential change in gene transcription with upregulation of inhibitory receptors including Lag3, TIGIT, Tim3 in addition to PD1 and CTLA4. Additionally, transcription factors c-Maf and Nfil3 were strongly upregulated consistent with their role in promoting IL-10 transcription in T cells.

In summary, soluble epitopes bind selectively to steady-state dendritic cells *in vivo* and promote differentiation of Tr1 cells in lymphoid organs (Fig. 3). The cells upregulate inhibitory receptors and switch transcription factor expression towards factors promoting IL-10 production. These cells traffic systemically where they are capable of suppressing activation of bystander cells through the IL-10 dependent downregulation of the antigen presenting machinery, primarily CD80 and CD86 expression, on antigen presenting cells [32]. In this way, if an apitope-induced Tr1 cell is specific for antigen A from within a tissue but the antigen presenting cell (APC) it associates with also presents
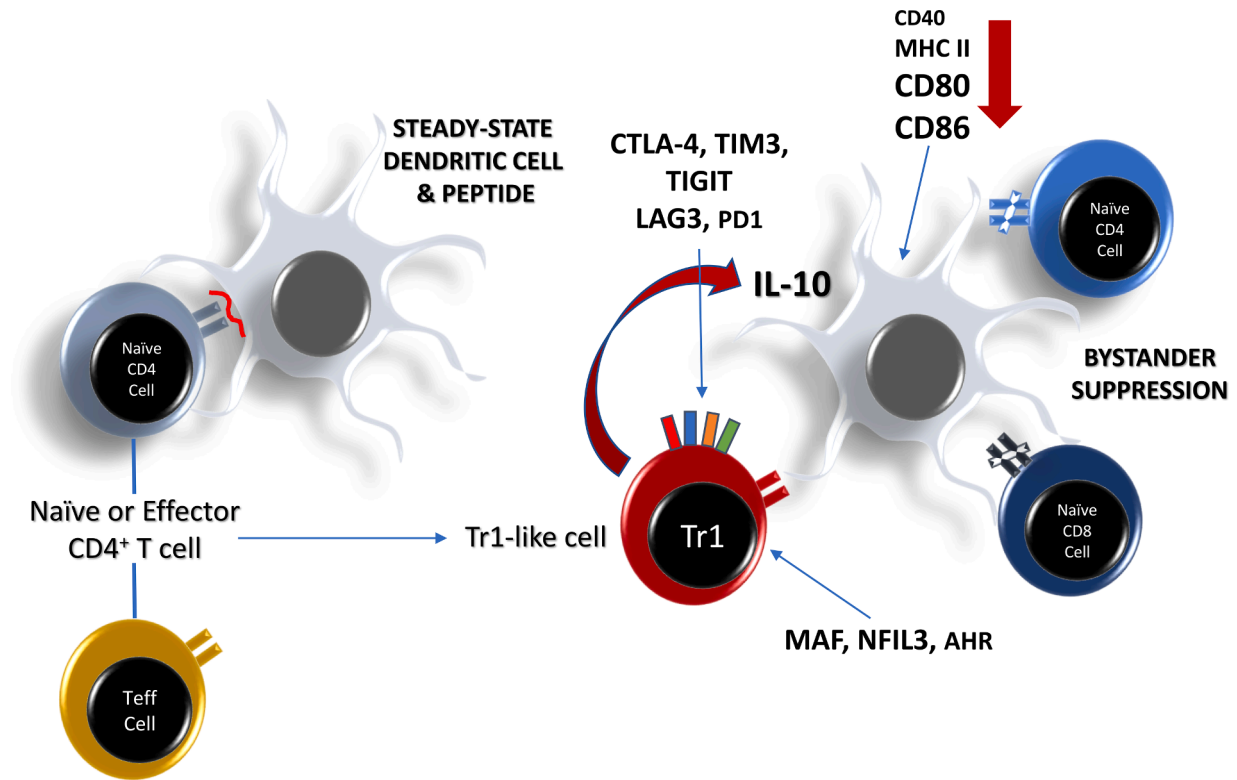
Fig. 3. Peptides designed to bind directly to MHC class II molecules without further antigen processing (antigen processing independent T-cell epitopes or apitopes) have been shown to bind selectively to steady-state dendritic cells in lymphoid organs following subcutaneous injection [32]. Repeated injection has a tolerogenic impact converting effector T cells into Tr1 cells. This conversion correlates with upregulation of inhibitory receptors and a shift in transcription factor expression towards IL-10 promoting factors MAF and NFIL-3. Tr1 cell recognition of their cognate antigen presented by other APCs results in secretion of IL-10 and down-regulation of the antigen presenting machinery of the APC (CD80 and 86) resulting in bystander suppression of CD4 and CD8 T cell responses to epitopes coincidentally presented by the same APC.
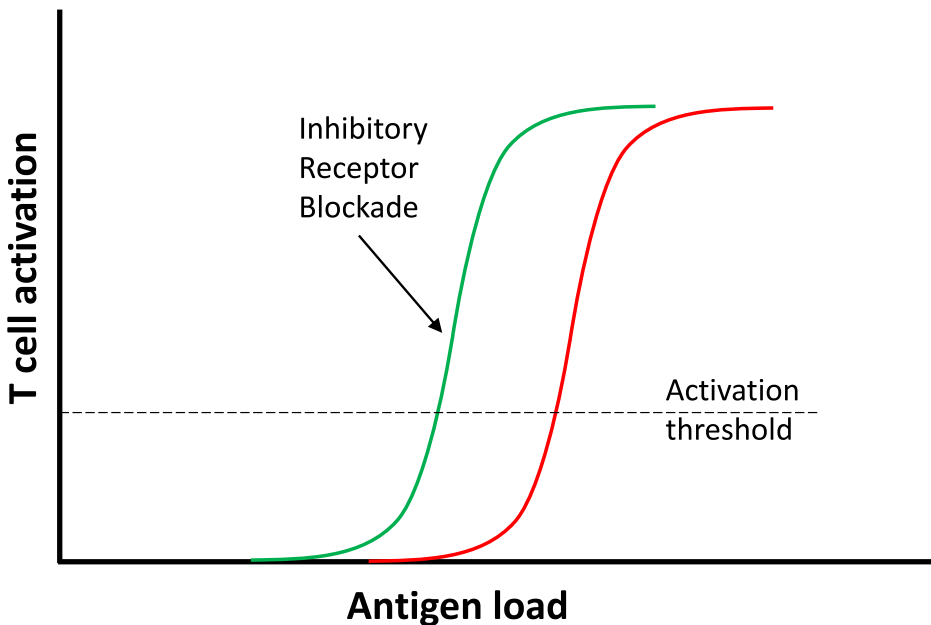

Fig. 4. Our recent work using Nr4a3 reporter mice has revealed a dose dependant negative feedback mechanism whereby administration of high dose of antigen effectively desensitizes individual T cells and this correlates with upregulation of inhibitory receptors [34]. A single high dose of antigen leads to upregulation of inhibitory receptors rendering the cells unresponsive to restimulation with low doses of antigen (red line). Inhibitory receptor blockade lowers the threshold for T cell activation in cells previously treated with a single high dose of peptide (green line). The difference in activation threshold illustrated here will vary according to the avidity of the TCR and potency of inhibitory receptor blockade.

antigens B, C, D and E from the same tissue then administration of an apitope from antigen A will drive IL-10 production leading to suppression of the antigen presenting machinery of the APC thereby mediating bystander suppression of T cells specific for the other antigens.

6. Molecular control of Tr1 cell differentiation in vivo

Consistent with the tunable activation threshold model of Grossman and Paul, we recently demonstrated how antigen and checkpoint receptor engagement recalibrate T cell receptor signal strength [34].
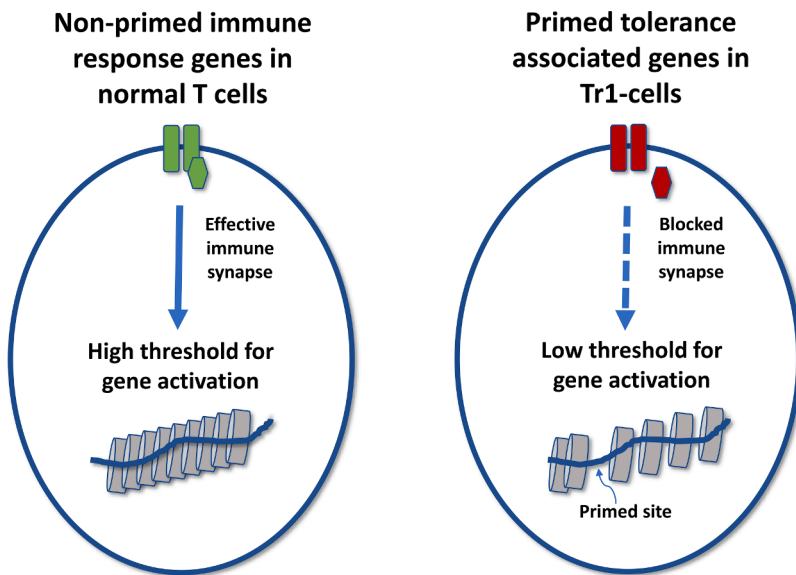
Fig. 5. Expression of inflammatory genes in non-tolerant CD4 T cells requires high levels of transcription factors in order to transcribe genes with a high threshold for gene activation. Our previous work has shown that soluble peptide treatment results in generation of Tr1 cells and a shift from expression of inflammatory genes to expression of a tolerance related gene signature [35]. This coincides with a membrane proximal block in cell signalling. Detailed analysis of the genes in the tolerance related signature revealed that they all show evidence of epigenetic priming. Tolerized T cells specifically maintain chromatin priming at a subset of primed DNase hypersensitivity sites within the archetypal T cell tolerance signature genes, allowing them to be activated at a signalling threshold below that of immune response genes. Epigenetic priming of selected, tolerance-associated genes enables their expression in anergic cells whose TCR mediated signalling has been markedly reduced.

Bending and colleagues have developed an Nr4a3 timer reporter bred onto the Tg4 background. Analysis of reporter-positive cells reveals dose-dependant induction of sets of genes such that low dose antigen triggers an activation module containing Nr4a3 and CD69 while an intermediate dose induces inflammatory cytokine expression and a high dose leads to induction of inhibitory receptors and a regulatory motif consisting of IL-10, Lag3, NFIL3 and TIGIT genes. Most importantly, this study shows that administration of soluble peptide at a specific dose prevents subsequent activation by lower doses of antigen, the cells are tuned not to reactivate when they see antigen 24 h after the first encounter. This correlates with upregulation of inhibitory receptors that were shown to be responsible for the recalibration/tuning effect of antigen encounter (Fig. 4).

The work described above explains how inhibitory receptors control T cell tuning in response to soluble peptide antigen. However, it is not clear how increasing doses of antigen leads to suppression of inflammatory cytokine production and the generation of a tolerogenic signature of gene expression including upregulation of inhibitory receptors and anti-inflammatory cytokines such as IL-10. Our recent work has shown that repeated exposure to soluble antigen induces a membrane proximal block in cell signalling [35]. Analysis of immune synapse formation showed that while T cell receptor molecules and the costimulatory molecule CD28 migrate into the immune synapse, downstream signalling molecules such as Zap70, Lat and PKC theta rapidly dissociate from the synapse hence disabling downstream signalling. How then does repeated exposure to soluble antigen induce anergy while at the same time promoting differentiation of IL-10 secreting Tr1 cells? These cells upregulate inhibitory receptors and yet remain responsive to antigen but with radically reduced levels of TCR signalling. Recently, Bevington and colleagues focused in on the genes upregulated in Tr1 cells and found that these genes were epigenetically primed. Epigenetic priming affects chromatin close to the genes in question and ensures that the specific site remains open, binding transcription factors such as ETS and RUNX1 and maintaining the associated gene in a semi-open state. Importantly, these genes are then able to transcribe in the presence of the low levels of transcription factors induced despite the membrane proximal block in cell signalling described above (Fig. 5). Importantly, this novel mechanism combining reduced cell signalling with epigenetic priming promotes the differentiation of cells capable of controlling immune pathology through a negative feedback mechanism involving selective transcription of anti-inflammatory genes.

7. Development of antigen-specific immunotherapy for treatment of autoimmune diseases

Our laboratory is currently developing the use of apitopes for treatment of autoimmune conditions. We have developed and conducted clinical trials of apitope therapy in Graves' disease and multiple sclerosis. Graves' disease arises through production of anti-thyroid stimulating hormone receptor (TSHR) antibodies, overstimulation of the thyroid gland resulting in overproduction of thyroid hormones and enlargement of the gland (goitre). Antithyroid drug treatment with carbimazole/methimazole is successful in approximately 50% of Graves' disease patients with the remainder requiring thyroid ablation by radioiodine treatment or surgery. In addition, up to half of Graves' disease patients develop Graves' eye disease resulting from an accumulation of fatty tissue behind the eye leading to ophthalmia. We have designed two apitopes from TSHR that were shown to suppress autoantibody production in an HLA-DR transgenic model of autoimmunity to TSHR [36]. This combination of peptides was then administered to individuals with mild to moderate Graves' disease in a phase I clinical trial. Each patient received an escalating dose of peptide (25, 50, 100, 400 and 6×800 μg) given intradermally at two-week intervals. Treatment was well tolerated with 10 of 12 patients completing the study. Improvement in thyroid function was observed in 7 of 10 subjects. Notably, there was a treatment-related reduction in serum TSHR autoantibodies that correlated directly with improvement in thyroid function. This promising outcome warrants further investigation in phase 2 trials.

Multiple sclerosis (MS) has a more complex immune pathology when compared with Graves' disease. The disease is associated with immune responses to at least three known major autoantigens (myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG)). Streeter and colleagues designed a cocktail of four dominant T cell apitopes derived from MBP [37]. ATX-MS-1467 was shown to suppress disease in a mouse model of MS expressing both disease-associated HLA-DR molecules and T cell receptors from an MS patient. Disease suppression was dose dependent and effective when given after disease onset. ATX-MS-1467 was shown to induce stable tolerance to MBP through induction of IL-10 secreting Tr1 cells [38]. Based on these results, we have completed three clinical trials including phase I trials in secondary progressive and relapsing remitting MS with a subsequent phase II trial in relapsing remitting disease [37, 39]. Intradermal treatment induced a significant decrease in new/persisting T1 gadolinium-enhancing lesions from baseline to week 16 of treatment,

Table 2

Known antigens and HLA-disease associations in classic polygenic autoimmune diseases.

Disease	HLA type	Relative risk	Antigen
Goodpasture's	DR2	15.9	Type IV collagen (a3)
Multiple Sclerosis	DR2	4.8	MBP, MOG, PLP
Graves' Disease	DR3	3.7	TSHR
Myasthenia Gravis	DR3	2.5	AChR
Systemic Lupus Erythematosus	DR3	5.8	dsDNA & Sm antigens
Primary Biliary Cirrhosis	DR8	3	Pyruvate dehydrogenase complex E2
Type 1 Diabetes	DR3/4 het	~25	Proinsulin, GAD, IA-2
Rheumatoid Arthritis	DR4	4.2	Citrullinated or carbamylated proteins
Pemphigus Vulgaris	DR4	14.4	Desmoglein, desmoplakin
Hashimoto's Thyroiditis	DR5	3.2	Thyroid peroxidase, thyroglobulin

returning to baseline values at week 48. This latter observation shows that the benefit from ATX-MS-1467 is transitory and implies that continuous treatment with peptides will be required to sustain suppression of disease. Importantly, safety data from 68 patients treated with ATX-MS-1467 shows no evidence of unexpected safety signals which means that prolonged treatment is justified and should be investigated. Table 2 lists a range of autoimmune conditions for which there is clear evidence of a disease-associated antigen and MHC-disease association. Each condition is suitable for treatment with apitopes based on their associated antigens. However, antigen-specific immunotherapies inducing Tr1 cells have been shown to inhibit responses to adjacent antigens via IL-10 dependant bystander suppression [32, 40–42]. In theory, it should be possible to design therapies based on an antigen from an inflamed tissue whether or not T cells specific for this antigen are directly linked to pathogenesis of the disease.

In summary, this article highlights the many advances in our understanding of immune regulation that have arisen from the predictions made by Grossman and Paul in 1992. The immune system is regulated by both intrinsic and extrinsic feedback mechanisms including activation induced cell death, inhibitory receptor regulated cell tuning, induction of regulatory T cells and extrinsic regulation by anti-inflammatory cytokines. Ultimately, this has led to the development of antigen-specific immunotherapeutic approaches suitable for treatment of a wide range of autoimmune conditions.

Acknowledgements

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