

REVIEW

Regulatory T-cell abnormalities and the global epidemic of immuno-inflammatory disease

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The incidence of autoimmune, allergic and inflammatory disease is increasing due to as yet unidentified environmental factors related to western living conditions. Here, I propose that alterations in the gut microbiome, acting via regulatory T cells (Tregs), may be responsible for this epidemic. Tregs control the threshold for peripheral antigen recognition via tonic downregulation of dendritic cell (DC) costimulation, and are also implicated in maintaining the tolerogenic function of DCs. In this model, minor perturbations in Treg number or function are predicted to lower the activation threshold, allowing proliferation and differentiation of self-reactive CD4T cells of too low an affinity to have undergone negative selection in the thymus. Failure to maintain the tolerogenic commitment of DCs exposed to commensal microbes and allergens could result in potentially pathogenic, allergic and inflammatory responses at epithelial surfaces.

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The incidence and prevalence of chronic non-infectious diseases related to the 'western' lifestyle continues to increase globally. Many of these diseases, which I will refer to as 'immuno-inflammatory' conditions, are caused by inappropriate immune responses to self or foreign antigens. They are usually considered under three distinct categories: true autoimmune diseases in which both the inducing and target antigens are self-derived, allergies in which both the inducing and target antigens are foreign and inflammatory diseases in which the inducing antigen is foreign but the response may induce pathology typical of autoimmune attack (such as celiac and inflammatory bowel diseases (IBD)). However, they share many common features that may provide useful clues regarding their aetiology and pathogenesis.

Some of these diseases were virtually unknown until modern times. Hay fever, for example, was described as most unusual in the early 19th century, but had become common in Europe and North America by 1900.¹ Type 1 diabetes (T1D), on the other hand, has been known since antiquity.² In the past 30 years the incidence of T1D is estimated to have risen at a rate of 3% per annum in the developed world.³ IBD, once a disease of Caucasians in the developed world, is now rapidly increasing in Asian populations in the Far East as living standards rise.⁴

GENES VERSUS THE ENVIRONMENT

The incidence of immuno-inflammatory disease has risen too rapidly to be attributable solely to genetic factors, although the latter are crucial in determining individual disease susceptibility. The most

influential genes are the MHC alleles first identified during the 1970's by means of linkage disequilibrium.⁵ Concordance for autoimmune disease between monozygotic twins living under similar environmental conditions is generally 30–40%, compared with 0–5% for dizygotic twins. No data regarding concordance between monozygotic twins living under highly disparate environmental conditions are available. The interaction between genes and environment is well illustrated in the changing epidemiology of T1D, in which the rising incidence is accompanied by earlier onset⁶ and a broadening of the gene subset associated with lifetime susceptibility.⁷ This pattern is consistent with a model in which the environment sets the threshold of genetic susceptibility.

Migration studies provide some of the best evidence of the strong influence of environment on disease incidence.⁸ Intriguingly, the strongest influence is birthplace, suggesting an environmental influence restricted to early life but with lifelong effects. The importance of early environmental effects is also evident in the data upon which Strachan⁹ based the 'hygiene hypothesis', proposed to explain increasing hay fever and eczema rates in post-WW2 UK. Strachan's analysis showed that the incidence of hay fever and eczema was related to birth order within the family: having older siblings had a protective effect, whereas having younger siblings was ineffective. Strachan postulated that 'allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings or acquired prenatally from a mother infected by contact with her older children. Over the past century, declining family size, improvements in household amenities and higher

standards of personal cleanliness have reduced the opportunity for cross infection in young families.⁹

Which infections are implicated in Strachan's birth order effect is a question that has intrigued both allergists and immunologists. The early studies of allergic (Th2-dependent) disease in humans attempted to explain susceptibility in terms of an abnormal Th1-Th2 balance resulting from lack of exposure to Th1-generating bacteria within the environment. However, susceptibility to T1D (a Th1-dependent disease) shows a very similar epidemiological profile to allergic disease, strongly suggesting that an alternative hypothesis is needed to explain environmentally mediated susceptibility. Abnormalities in regulatory T cells (Tregs), which are capable of controlling a full range of immune functions, are a more likely common cause of the multiple subtypes of immuno-inflammatory disease.

Evidence is now accumulating in support of a model in which the gut microbiome is the crucial environmental factor responsible for susceptibility to immuno-inflammatory disease.^{10–13} Many aspects of gut microbiome biology dovetail neatly with the migration effect and the hygiene hypothesis. The gut is the site of the great majority of interactions between microbes and the immune system. Gut commensal microbes are established in early infancy and maintained throughout life in an essentially stable symbiotic relationship,¹⁴ explaining how infectious events early in life can produce life-long effects on the immune system.

IMMUNE MEDIATORS OF ENVIRONMENTAL EFFECTS

In order to understand how the gut microbiome could impact on immuno-inflammatory disease, it is necessary to understand the immunological factors involved in disease initiation and progression. The discovery of natural Tregs by Sakaguchi¹⁵ provided for the first time a mechanism that could explain how susceptibility to spontaneous chronic autoimmune and immuno-inflammatory diseases arises in otherwise normal individuals. Prior to that time, models of autoimmune disease often relied on immunisation with self-antigen plus adjuvant, which usually produced self-limiting disease unless combined with lymphopaenia-inducing manipulations such as removal of the thymus combined with irradiation.¹⁶ These early models focussed attention on infectious agents as triggers of disease. Infections were hypothesized to activate self-reactive cells by means of molecular mimicry and these cells were then believed to propagate an ongoing autoimmune attack. However, these models could not explain why the infectious disease burden was inversely related to the prevalence of immuno-inflammatory disease in the developed world, why such a small proportion of infected people developed a particular autoimmune disease and why those affected by sporadic autoimmunity showed no evidence of lymphopaenia.

Much more compelling was a model in which a network of Tregs was proposed to act throughout life to prevent or control autoimmune responses.¹⁷ If and when the network failed to perform at peak level, the individual would become susceptible to the development of those diseases to which they had a genetic predisposition. The onset of such diseases would not necessarily require an infectious trigger, but the overall lifetime probability would be low unless a genetic mutation produced a major disruption to Treg function, as, for example, the FoxP3 mutations that lead to the IPEX syndrome.¹⁸

HOW DO TREGS PREVENT AUTOIMMUNE DISEASE?

A plethora of mechanisms has been proposed to account for Treg activity *in vivo* and *in vitro*.¹⁹ Although Sakaguchi's original studies

indicated that Tregs prevented even the earliest stages of autoimmune disease, the mechanistic focus soon shifted to suppression of ongoing T-cell proliferation *in vitro*.²⁰ The vast majority of mechanistic studies have focussed on the ability of Tregs to downregulate responses after initiation, usually via direct cell–cell contact with proliferating T cells. Thus, *in vitro* tests use a stimulus, usually an anti-CD3mAb, to generate a naive T-cell response, which is then suppressed by Tregs. *In vivo* experiments use adoptive transfer of Tregs to suppress ongoing responses, or measure the outcome of Treg deficiency or depletion in terms of inflammatory responses. These experiments have been interpreted as indicating that the ratio of Tregs to conventional T cells is the crucial determinant of autoimmune susceptibility.

This interpretation is not compatible with the original experiments of Nishizuka and subsequently of Sakaguchi, which demonstrated that induction of non-specific CD4T lymphopaenia by a number of different means induced the same spectrum of autoimmune diseases that were produced by adoptive transfer of Treg-depleted cells to lymphopaenic animals.^{21,22} During CD4 lymphopaenia, the ratio of Tregs to conventional T cells remains unchanged,²² reflecting the dependence of Tregs upon IL-2 made by conventional T cells. Thus susceptibility of lymphopaenic animals to autoimmune disease is due to an absolute rather than relative deficit in Tregs. These data suggest that the direct target of Tregs is a cell population whose number is not affected by non-specific CD4T lymphopaenia. An obvious candidate is the dendritic cell (DC), which interacts specifically with Tregs via self-peptide-MHC II complexes that serve as the ligands of Treg TCRs. This conclusion is supported by direct *in vivo* evidence, obtained using intravital microscopy, of Treg interactions with DCs but not 'target' T cells.^{23,24}

In vitro studies have demonstrated a direct effect of Tregs in reducing expression of the costimulatory molecules CD80 and CD86 on the surface of DCs.^{25,26} Tregs have also been shown to prevent the upregulation of costimulatory molecules during maturation/activation of DCs.²⁷ Ongoing studies in my laboratory (described below) have shown that Tregs downregulate steady-state DC expression of costimulatory molecules *in vivo*. This means that they control the threshold for low affinity peripheral antigen recognition, as signals mediated via costimulatory molecules are vital in driving activation and proliferation when TCR signals are of very low avidity.

One of the clearest illustrations of the profound effect of Tregs on low affinity CD4T cell responses *in vivo* is their effect on fast phase lymphopaenia-induced proliferation (LIP).²⁸ Fast phase LIP is absolutely dependent on TCR specificity and involves only 4–6% of naive CD4T cells in C57Bl/6 mice.²⁹ Moreover, fast phase LIP is a necessary precursor to lymphopaenic immuno-inflammatory disease, as it converts naive CD4T cells to effector cells that can enter non-lymphoid tissues.²⁹ To understand how 4–6% of naive CD4T cells are recruited into fast phase LIP in lymphopaenic hosts and yet do not divide in lymphosufficient mice, we compared expression of costimulatory molecules by DCs in lymphopaenic and lymphosufficient animals. Not only did DCs express higher levels of costimulatory molecules in lymphopaenic animals but full reconstitution with purified Tregs supported by administration of exogenous IL-2 normalised DC costimulation levels. Treg-mediated control of costimulation had the predicted effect of abolishing fast phase LIP and the ensuing immuno-inflammatory disease. In contrast, reconstitution with conventional CD4T cells increased both costimulation and fast-phase LIP. These results strongly suggest that control of steady-state DC costimulation is a function of Tregs and that control of DC-stimulatory capacity is the first point at which Tregs act to prevent autoimmune disease.

EFFECT OF TREG ABNORMALITIES ON IMMUNO-INFLAMMATORY DISEASE—A THRESHOLD MODEL

By modulating DC costimulation, Tregs would effectively control the threshold for low affinity antigen recognition (Figure 1). This has important implications for how Treg deficiencies could lead to susceptibility to autoimmune disease. Negative selection in the thymus removes only the highest affinity anti-self T cells. As mentioned above, the existence of self-reactive specificities within the normal T-cell repertoire can be demonstrated under a range of abnormal experimental conditions, but is not evident otherwise, indicating that the threshold for responding to self-antigen is not reached in normal animals under normal conditions. However, if costimulation increases because of a Treg deficit, it would be possible for those autoreactive cells that are closest to the threshold to receive a sufficiently strong signal to drive proliferation and differentiation, necessary first steps in a pathogenic autoimmune response. The specificity of these autoreactive cells would be affected by the genotype of the individual, which would control the expression and processing efficiency of self antigens, their affinity for self MHC and the likelihood that suitable self-reactive T cells would be selected in the thymus. As the Treg deficiency became more severe and the threshold dropped further, a larger number of low affinity autoreactive cells would fall within the window of activation. This model explains how the incidence of spontaneous autoimmune disease would rise as defects in the Treg compartment became more frequent or more severe, why the average age of onset would decrease and why additional disease susceptibility genotypes would be involved as the threshold was lowered.

It is important to note that, according to this model, autoimmune disease is not caused by an aggressive, high affinity response but instead by a far more indolent, low affinity response. The capacity for severe tissue damage is due not to the strength of the immune attack but rather to the inability of the immune response to clear the autoantigen, leading to chronic inflammation. The Treg abnormalities that allow such responses to start may also be apparently insignificant, just as long as they allow enough cells to differentiate, enter the target tissue and set up an ongoing feedback loop of antigen liberation and triggering of effector cells. Human autoimmune disease can initiate many years before it manifests clinically, as indicated by the presence of autoantibodies in otherwise well subjects that later go on to develop disease. This in itself indicates the indolent nature of the autoimmune response.

Control of the activation threshold by Tregs applies to anti-foreign as well as anti-self responses. Essentially harmless foreign antigens from non-microbial sources, unaccompanied by microbial adjuvants, such as TLR ligands, may effectively be ignored by the immune system under normal conditions. Interestingly, when inhaled and ingested antigens do stimulate pathogenic immune responses, they are generally Th2-biased, suggesting a relatively low-level immune stimulus.³⁰

IS THRESHOLD CONTROL THE ONLY PROBLEM LEADING TO IMMUNO-INFLAMMATORY DISEASE?

In normal animals, even high affinity anti-self T cells are deleted in the periphery, as indicated by a large number of studies in TCR transgenic models.³¹ Thus, the threshold model presented above can explain only one part of the puzzle. Our recently published demonstration that some DCs subsets may subserve purely tolerogenic functions under normal circumstances³² provides a clue as to how interactions with high affinity antigen can change during Treg deficiency.

We have shown that Langerhans cells in the epithelial layer of the skin induce tolerance and suppress the response of the deeper dermal DCs.³² This finding is of immediate importance to our understanding of why skin commensal microbes living in the superficial epithelium do not stimulate an inflammatory response, despite expressing an essentially indistinguishable array of TLR ligands from pathogenic organisms that invade through the epithelial basement membrane and are thus presented to T cells by immunogenic dermal DCs.

Langerhans cells may be the first to be identified of a group of pre-committed tolerogenic DCs. I have postulated that such DCs are present in the steady state in all secondary lymphoid organs and are responsible for reinforcing self-tolerance by deleting peripheral T cells responding to self antigens.³¹ These tolerogenic DCs are likely to be responsible for peripheral deletion in response to intravenous antigen. Interestingly, peripheral deletion is abolished under conditions of lymphopaenia,³³ suggesting that Tregs may also be responsible for maintaining tolerogenic commitment in DCs. Treg deficiency would not only interfere with deletion of self-reactive cells that react in response to a lowering of the peripheral antigen recognition threshold but would also prevent induction of tolerance to commensal organisms at epithelial surfaces, leading to inflammatory conditions such as IBD.

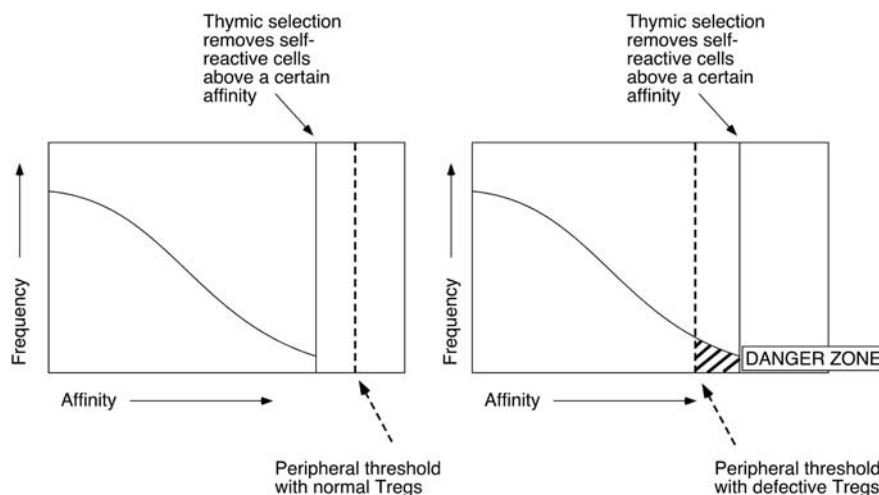


Figure 1 A model illustrating how Treg-dependent decreases in the peripheral T-cell activation threshold expose an increasingly large subset of potentially autoimmune cells to activating signals. Left panel: healthy Treg number and function; right panel: defective Treg number or function.

DO TREGS INTERACT WITH THE MICROBIOME?

Several recent studies have provided direct evidence that gut bacterial products can induce Tregs.^{13,34,35} It is not yet clear how these Tregs function *in vivo* and whether they recirculate in a manner that is consistent with the postulates of the models presented above. A second possibility is that survival and function of thymically derived Tregs is dependent on IL-2 produced as a result of immune responses driven by gut microbes.

As the entire gut microbiome functions as a complex ecosystem, it is unlikely that a single microbial species or property will determine all its immunological effects. The slow but inexorable change in autoimmune disease incidence³⁶ suggests incremental changes in the environmental influences that generate disease susceptibility. In each new generation, a less complex gut microbiome may be established, as conditions of birth and early life allow less and less contact with a wide variety of potential gut microbes. Clear differences in gut colonisation have been described for babies born under different socioeconomic conditions¹¹ or by caesarian section.³⁷

THE FUTURE

While the increasing incidence of immuno-inflammatory disease is alarming, its dependence on environmental factors is heartening, as it suggests that the trend will be fully reversible once we find out which microbial factors have been lost. It will be also be important to determine whether any of the other chronic diseases of the western lifestyle also have a cryptic immune basis. Evidence is accumulating that Treg abnormalities are involved in the pathogenesis of vascular disease,³⁸ obesity³⁹ and the metabolic syndrome.⁴⁰ World Health Organisation involvement in an ongoing global initiative to combat non-communicable diseases⁴¹ underscores the importance of an integrated approach to these preventable diseases.

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