

The Impact of Infection on the Incidence of Autoimmune Disease

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Abstract: Falling infection rates in the developed world are being matched by a rapidly rising incidence of allergic and autoimmune diseases. This review explores the hypothesis that there is a causal link between these phenomena and that infections can prevent the onset of autoimmune disease. The hypothesis is discussed with particular reference to Type I diabetes in the NOD mouse and the ability of the helminth infection *Schistosoma mansoni* to prevent its onset. The article addresses the possible mechanisms that underly this protection. The effects of protective pathogen-derived agents on key cells of the innate immune system such as dendritic cells are distinct and include the production of anti-inflammatory cytokines such as IL-10. The most likely mechanisms by which these innate changes prevent the subsequent adaptive autoimmune destruction are: (1) the production of systemically high levels of cytokines that oppose the production of cytokines that drive the autoimmune process – possibly via the action of natural killer T (NKT) cells (2) the induction of regulatory T cells that inhibit the action of autoreactive cells and (3) the production of pathogen-specific T cells that are not autoreactive and compete with autoreactive cells for survival signals such as cytokines and T cell receptor ligation.

INTRODUCTION: BY WHAT MECHANISMS MIGHT INFECTION PREVENT AUTOIMMUNITY?

The vertebrate immune system is designed to combat infection while remaining tolerant of self. In the last fifty years, there has been a fall in the prevalence of serious childhood infections such as tuberculosis, diphtheria and hookworm in the developed world. Many factors may account for this trend, including better housing conditions, improved sanitation and the nutritional status of the population. Vaccination programmes against serious childhood diseases also play an important role. It may, however, be premature for industrialised nations to congratulate themselves too heartily on their successful assault on infection because there is a growing suspicion that the devil has found work for the immune system's idle hands.

Falling infection rates have been matched by a concomitant rise in the prevalence of autoimmune diseases such as type I diabetes (1) please see Instructions for Authors for presentation of references in text and rheumatoid arthritis and also in allergic diseases such as asthma and eczema (2,3), in these same industrialised countries. By contrast, in the developing world, where the prevalence of childhood infection has been stable, the incidence of autoimmunity is much lower and has also remained stable. The recently described "hygiene hypothesis" has discussed the notion that changes in living conditions and infection rate may account for the increased prevalence of allergy. Now it has been postulated that the reduced incidence of infection, particularly parasitic infection is a causative factor in the rapidly rising incidence of the major autoimmune diseases. The development of autoimmune diseases is governed by

complex genetic susceptibility factors (4,5) and environmental modifiers in both mouse and man. The importance of environmental factors in autoimmune disease can be seen in the low concordance rates between monozygotic twins for some diseases. For instance, monozygotic concordance in diabetes mellitus is only 40%.

This review will address the evidence that infection is a key environmental factor that can protect individuals from autoimmune disease and will examine the mechanisms that may account for the protection observed in some systems. In particular, the ability of parasitic worms such as *Schistosoma mansoni* to halt the autoimmune process will be explored. Much of the discussion of autoimmune disease will focus on type I diabetes mellitus. This disease is extremely easy to diagnose, therefore the observed increase in incidence is very unlikely to be due to improved detection. In addition, the NOD mouse model of diabetes mellitus closely mimics the human disease and therefore many clues to the pathogenesis of disease and mechanisms of protection can be obtained from this model.

EVIDENCE THAT INFECTION PREVENTS AUTOIMMUNITY

A wealth of anecdotal evidence suggested that infection could prevent autoimmunity in animal models before the hypothesis was formally investigated. The outbreak of infections such as pinworm and murine hepatitis virus in the animal houses of research facilities was often accompanied by a fall in autoimmune disease incidence in autoimmune-prone mouse strains such as the non-obese diabetic (NOD) mouse.

The relationship between infection and autoimmunity has now been investigated by a number of groups using the NOD mouse as a model for human autoimmune disease. A proportion of NOD mice spontaneously develops diabetes from twelve weeks of age. This autoimmune disease results

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from the destruction of the beta cells of the pancreas by cells of the immune system. Disease prevalence is roughly 80% in females and 20% in male mice at thirty weeks of age.

It was subsequently shown that inoculation of NOD mice with complete Freund's adjuvant (CFA) (6) or the bacillus Calmette-Guerin (BCG) vaccine (7,8,9,10) could protect NOD mice from diabetes mellitus.

A later study in the NOD mouse (11) showed that the development of autoimmune diabetes could also be inhibited by infection with the helminth *S. mansoni* or its eggs. *S. mansoni* was chosen in this study because it was clear that the immune responses caused by infection with this helminth could influence the subsequent responses to other infections or antigens (12). The protection from diabetes mediated by *S. mansoni* is only observed if initiated when the mice are approximately four weeks of age. No protection was observed if treatment is started in eight-week old mice.

A subsequent experiment showed that injecting four week old mice with two extracts derived from the helminth, soluble worm antigen (SWA) and soluble egg antigen (SEA) could completely protect mice from diabetes (Zaccone *et al* 2003 – *EJI*(5) 1439-49.) Soluble worm antigen and soluble egg antigen are made by homogenising worms or eggs from the helminth and removing the particulate matter. Clearly then, they contain potent immunomodulatory products; where they exert their effects will form much of the focus of this review.

THE EFFECTS OF INFECTION ON THE INNATE AND ADAPTIVE IMMUNE SYSTEMS

The protection from autoimmunity that certain infections appear to mediate throws up the intriguing possibility that we may one day be able to protect individuals from autoimmunity by employing immunomodulatory compounds derived from pathogens. In order to accomplish this, it is important to understand more precisely the mechanisms that cause infections to halt the damaging autoimmune responses. The genetic pre-disposition to autoimmunity is complex and incompletely understood. One would not, for instance, want to vaccinate the population against one autoimmune disease only to precipitate another. This possible pitfall was highlighted in a study by Baxter *et al.* (13). They showed that a single dose of heat-killed bacillus Calmette-Guerin (BCG) administered intravenously to 8-week-old NOD mice prevented diabetes but precipitated a syndrome similar to systemic lupus erythematosus (SLE.) Treated mice developed haemolytic anaemia, anti-DNA and anti-nuclear autoantibodies and an increased severity of sialadenitis. This data reminds us that a clearer understanding of genetic predisposition to autoimmunity is necessary before therapies derived from pathogens could be used in the clinical setting.

Immunity to infection is mediated by the innate immune system, which responds quickly to limit the severity of the infection in the initial stages and the adaptive immune system, which responds more slowly but mounts a response that generates memory and specifically targets the individual pathogen and is therefore more efficient. The cells of the innate immune system are not merely cannon fodder, however in the war of attrition that characterises early

immune responses. They are also sophisticated sentinels that play a key role in the development of an appropriate adaptive immune response

This theme of the instructive role of the innate immune response in determining the phenotype of adaptive immune responses has been at the forefront of immunology in recent years (14). The paradigm developed because of the limitations of the theory that the only determinant of the initiation of an adaptive immune response is whether T cells encounter self or non-self antigen on the surface of antigen presenting cells.

It is now clear that not all potential antigens elicit an immune response. For instance, a large proportion of the world's population consumes animal and plant proteins in their diets but do not make immune responses to them. Many vaccines composed of non-self proteins will not induce protective immunity unless other substances known to promote inflammation (adjuvants) are added. In addition, processes such as puberty and pregnancy inform us that what constitutes "self" is dynamic rather than static. T cells, though famed for their specificity, exhibit a significant degree of degeneracy: cross-reactivity with self-antigens therefore exists. It had also become clear that as well as TCR-MHC interactions (signal 1), further receptor-ligand interactions between APCs and T cells, known as "co-stimulation" were also necessary for T-cell activation.

These issues led Janeway (15) to propose the existence of conserved structural features of pathogens that were not present on host cells. He believed that these pathogen-associated molecular patterns (PAMP's) would bind to pattern recognition receptors (PRR) on antigen presenting cells and drive a signalling cascade that would allow them to provide the second signal. In this way, peptides presented to the adaptive immune system in the presence of a PAMP would drive an immune response whereas peptide alone would not. This model attracted a wealth of support but the existence of the PRR remained unconfirmed until 1997 when the human Toll-like receptors (TLR) were discovered (16). Ten members of this family have now been identified, all of which recognise conserved epitopes on micro-organisms (17). The corollary of the notion that innate immunity drives adaptive immunity is that it almost certainly has an integral role in driving autoimmunity. If then, infection modifies the incidence of autoimmunity it probably does so, at least in part, through actions on the innate immune system. Therefore, an understanding of the interaction of pathogens with antigen-presenting cells is key to understanding both immunity and autoimmunity. The effects of some of the infections that prevent autoimmunity on APC's will also be discussed.

The most efficient antigen-presenting cells both *in vivo* and *in vitro* are dendritic cells (DC's.) Significantly, they are the only professional antigen presenting cell that can provoke autoimmune disease when transferred from donors that are undergoing autoimmune attack to unwitting recipients (18). Dendritic cell transfer can also *prevent* autoimmune diabetes in the NOD mouse (19.) Their key role at the interface of immunity and tolerance has been recently emphasised by studies that suggest that they are essential for

establishing peripheral tolerance of autoreactive T cells that have escaped thymic deletion through peripheral deletion, anergy and the induction of regulatory T cells (20)

So what features bestow such powerful properties upon dendritic cells? DC's are a heterogeneous population of bone marrow derived cells that circulate between tissues and blood. They are particularly abundant at interfaces between the host and its environment – for instance beneath the epidermis of the skin and the respiratory epithelium. DC possess abundant phagocytic receptors and can capture antigens from their immediate environment. Following this, they migrate to secondary lymphoid organs and present antigens to T cells in association with classical and non-classical MHC molecules.

In response to some stimuli such as infection, DC's undergo a profound phenotypic change, described as activation or maturation that affects the response of the T cells with which they interact (21).

Upon activation DC's lose their ability to internalise antigen. They become stellate in shape and can interact with many T cells. They up-regulate expression of MHC class I and II molecules and present on them peptides that they have taken up and processed. They increase their surface expression of co-stimulatory molecules such as CD40, CD80 and CD86 (22) and produce cytokines and chemokines. This "classical" activation pathway is generally mediated by the Toll receptors whose existence Janeway had predicted.

This represents an elegant way in which the immune system might discriminate between harmful and non-harmful situations.

When BCG is incubated with murine dendritic cells, this process occurs. The DC's produce IL-12 and IL-10 and up-regulate MHC class II and co-stimulatory molecules following incubation with BCG. (23) However, not all infectious products that influence the interaction of DC with T cells activate the DC in this way. Some antigens derived from microbes seem to have little effect of the expression of co-stimulatory molecules and the production of cytokines by the dendritic cells. However, when DC's that have been incubated with such products are allowed to present antigen to T cells, an adaptive immune response is initiated and the T cells appear to be functionally polarised.

Soluble egg antigen (SEA) derived from *S. mansoni* has no effect on DC's when incubated with them alone *in vitro*. Nonetheless, murine DC that have been treated with SEA can induce polarised adaptive immune responses (discussed below) that could potentially oppose the damaging autoimmune responses seen in diabetes mellitus (24,25). Products such as soluble egg antigen and ES-62 (the excretory secretory product of the helminth *A. Viteae*) (26) that clearly affect the key antigen presenting cells but do not elicit obvious phenotypic changes in the dendritic cell prior to their interaction with T cells have been described as "conditioning" dendritic cells. (27)

The fact that SEA is affecting dendritic cell function can also be seen in its effects on LPS-mediated maturation of dendritic cells. SEA reduces the levels of IL-12 secretion normally induced by LPS and increases the IL-10 secretion that this compound normally elicits (Zaccone *et al* 2003 –

EJI(5) 1439-49) Our studies did not show an effect of the other protective schistosomal antigen SWA on dendritic cells in the NOD mouse. The potentiation of IL-10 secretion that is mediated by soluble egg antigen of *S. mansoni* is potentially a very significant finding because of the association of IL-10 with the generation and effector function of regulatory T cells – a topic which is discussed in more detail later.

It is clear therefore that BCG and SEA, which both protect NOD mice from diabetes have distinct effects on DC. No unifying mechanism of protection is immediately apparent, therefore we must consider the effects of such infections on the adaptive immune system, both directly and indirectly via their effects on DC.

Th1/Th2 Deviation as a Mechanism of Protection

The effects of infectious products on the DC determine whether T cells proliferate and also the type of adaptive immune response that occurs. The phenotype of the adaptive immune response is controlled by CD4+ T cells. CD4+ T cells can be broadly divided into two functional subsets (Th1 or Th2) based on the cytokines that they secrete (28)

The factors that influence whether a T cell becomes polarised towards the Th1 or Th2 phenotype at the initiation of an adaptive immune response include the cytokines secreted by the APC, the concentration of antigen presented at the cell surface and the co-stimulation that the APC provides. Th1 cells secrete mainly interferon- γ (IFN- γ) and orchestrate cell-mediated responses. By contrast, Th2 cells make IL-4. They drive antibody-mediated immunity that is best suited to the elimination of extracellular pathogens and promote the production of certain antibody isotypes. Th1 cells antagonise the development of Th2 cells from non-polarised T cells and vice-versa. They are in, effect, an immunological yin-yang. Many mechanisms probably account for this antagonism but one of the best characterised is the differential effects of IFN- γ and IL-4 on the IL-12 receptor. IFN- γ stabilises cell surface expression of the IL-12 receptor thus promoting the production of more IFN- γ . IL-4 however, downregulates the expression of the IL-12 receptor on T cells thus preventing the development of Th1 cells. Therefore, immune responses to certain pathogens become polarised towards cell-mediated or antibody mediated immunity depending on the conditions in which naïve T cells become activated.

The autoimmune destruction of the beta cells of the pancreatic islets of Langerhans seen in diabetes is driven both in mice and humans by CD4+ T cells. The CD4+ T cells that mediate islet destruction in type I diabetes are polarised towards the Th1 profile. The immune response to *S. mansoni* in mice and humans is initially Th1-polarised but becomes a Th2 response when the worms lay eggs (29). These Th2 responses are characterised by production of IgE antibodies, eosinophilia and Th2 cytokines such as IL-4, IL-5 and IL-13. This correlates well with the tendency of SEA to downregulate production of IL-12 by dendritic cells, as this cytokine potently drives Th1 responses.

Therefore, is *S. mansoni* protective because the Th2 immune response to the parasite antagonises the development of the pathogenic Th1 cells? It is certainly true that in

the model of schistosomal protection from diabetes outlined above, *in vitro* re-stimulation of spleen cells from treated mice with schistosomal antigens results in high levels of IL-4, IL-5, IL-10 and IL-13, which can be described as a Th2-type response. It is also known that these cytokines are produced by T cells because depletion of T cells before re-stimulation of the splenocytes with antigen abrogates cytokine secretion, whereas depletion of B cells does not.

Normally, splenocytes from NOD mice that are transferred to SCID recipients cause rapid induction of diabetes; however splenocytes from mice that have been treated with *S.mansoni*, soluble worm antigen (SWA) or soluble egg antigen (SEA) have a much reduced capacity to transfer disease to SCID recipients (Zaccone et al. *manuscript submitted*.) This would be consistent with a skewing of islet-specific immune responses towards a Th2 profile but there are other feasible explanations.

The nature of the T cells that orchestrate these Th2 responses is also a point of contention because it has been previously shown that NOD mice are deficient in a subset of T cells called Natural Killer T cells (NKT) (30). These T cells, which also carry some markers of NK cells recognise glycolipids associated with the non-classical MHC class I molecule CD1 on antigen presenting cells. The most well characterised subset of NK T cells express the invariant V α 14J α 18 T cell receptor. They can produce both IFN- γ and IL-4 and are therefore thought to be capable of mediating both Th1 and Th2 responses. The V α 14J α 18 subset bind very strongly to the glycosphingolipid, alpha gal ceramide in association with CD1d. This substance is found in marine sponges and it is thought that it is probably similar to a host molecule that the NKT cells recognise. Recognition of alpha-gal-ceramide by the T cells is associated with IL-4 production (31)

Interestingly, numerous studies have shown that both NOD mice and diabetic humans are deficient in NKT cells (32-35) The proportion of NKT-like thymocytes is decreased in NOD mice aged 3-5 weeks of age, which is just before the mice develop insulinitis. This defect is corrected in the thymus by 10 weeks of age but persists in the spleen. Transfer of cell populations that have been enriched for NKT cells can reduce disease incidence. The forced expression of a V α 14J α 18 transgene (36), which increases the number of NKT cells also protects the mice from diabetes as does treatment with their natural ligand alpha galactosyl ceramide (37,38). By contrast, crossing NOD mice onto a CD1d $^{-/-}$ background (so that NKT cells cannot recognise their natural ligand) accelerates disease. The protection that NKT cells appear to afford the NOD is associated with high levels of Th2 cytokines such as IL-4, much like the protection associated with *S.mansoni* treatment. Intriguingly, experiments carried out by our group show that treatment of NOD mice with SWA protects mice from diabetes and also significantly increases the number of NKT cells in the livers of treated mice.

But is Th2 skewing through the expansion of previously rare NKT cells the reason that *S.mansoni* protects NOD mice from diabetes or simply a co-incident observation?

Certain other experimental models support the notion that deviation of immune responses from a Th1 to a Th2 phenotype may be a mechanism by which NOD mice are protected from diabetes. NOD mice that transgenically express the signature Th2 cytokine IL-4 in the pancreatic islet cells (39) are protected from diabetes. There are, however, convincing arguments that deviation towards Th2-polarisation cannot explain every case of infection protecting NOD mice from diabetes. It has been argued that in many cases where protection is accompanied by high levels of Th2 cytokines, the cytokines are simply associated with protection rather than a causative factor. (40)

DC treated with BCG, which is also protective against diabetes drive a Th1 response when co-cultured with T cells, as expected by their tendency to provoke IL-12 production from DC's. Furthermore, inoculation of mice with BCG causes Th1 responses characterised by high levels of IFN- γ and low levels of IL-4.

In addition, in the BCG-induced protection model, Serreze and colleagues have shown that protection can still occur in IL-4 and IL-10 knockout mice on a NOD background and NOD mice that have the IL-4 or IL-10 genes knocked out do not have accelerated diabetes. Furthermore, there is no protection from diabetes in IFN- γ knockout mice, indicating that this Th1 cytokine is necessary for BCG to mediate its protective effects.

Immune Regulation as a Mechanism of Protection

It is clear that *S.mansoni* infection does promote Th2 responses in the NOD mouse but it is now apparent that there may be other generalised effects on the immune system following parasitic infection that could also account for the observed protection from diabetes. Furthermore, infections that do not cause Th2 deviation can also protect from diabetes. Attention has therefore focused on the ability of infection, particularly parasitic infection to induce regulatory T cell populations.

The existence of regulatory T cells that suppressed the actions of other T cell subsets was first posited as a mechanism that might maintain tolerance in the early 1970's. However, a lack of consistent evidence for their existence led to declining interest in the field. Since the mid 1980's, however, regulatory T cells have undergone something of a renaissance and evidence for their existence has been growing since that time (41)

So what is the evidence for policing of T cells by other T cells as a mechanism by which tolerance is maintained? For a start, it is clear that T cells are somewhat degenerate in their specificity for antigen and deletion of autoreactive T cells in the thymus (which is known as central tolerance) cannot eliminate all autoreactive cells T cells from the repertoire.

Therefore, even in normal healthy individuals, a proportion of peripheral T cells are autoreactive but most individuals do not suffer from autoimmune diseases; therefore there must be extrathymic or "peripheral" mechanisms for maintaining tolerance. There are many proposed mechanisms of induction of peripheral tolerance including immunological ignorance of the antigen, anergy (unresponsiveness) upon

encountering self-antigen and deletion upon encountering self-antigen. It is likely that anergy and deletion in response to self-antigen occur when the antigen is presented in the absence of appropriate co-stimulation, which the presence of infection would facilitate.

An intriguing finding though, is that in many systems, tolerance can be adoptively transferred between animals by T cells – suggesting the possibility of *active* suppression of the activity of autoreactive cells. In addition, Sakaguchi and colleagues (42) showed that an athymic mouse (i.e. lacking T cells) that was reconstituted with T cells depleted of CD25+ cells developed multisystemic autoimmune disease. This was the first investigation to propose a surface marker that could identify regulatory T cells. Subsequently, the expression of other markers such as CD45RB have been proposed to divide regulatory and non-regulatory populations.

Further investigation of these phenomena was carried out in a number of *in vitro* and *in vivo* systems. *In vitro*, CD4+ CD25+ cells are able to suppress by 80 or 90% the proliferation of CD4+ CD25- T cells, when both T cell subsets are stimulated by antigen (43). In these *in vitro* systems, suppression of proliferation appears to be cell-contact dependent. *In vivo*, however, in models in which the CD4+ CD45RB^{low} subset was shown to protect mice from inflammatory bowel disease (44,45) protection was dependent upon the soluble factor TGF- β . In a further study Groux *et al.* generated T cell clones that could prevent inflammatory bowel disease via TGF- β and IL-10 (46,47). Furthermore, TGF- β and IL-10 knockout mice develop multi-systemic autoimmune diseases. Therefore, the mechanisms by which these cells mediate suppression of T cell proliferation and autoimmune diseases appear to vary in different experimental systems.

It seems that regulatory T cells can be generated in the thymus (48) or the periphery (49) but the exact antigen specificity of regulatory T cells is still not clear in most of the models described.

Therefore, evidence for the presence of regulatory cells under normal physiological conditions has accumulated, though much about their function remains obscure. An attractive theory is that infection induces regulatory cells as a way of limiting immune-mediated damage of normal tissues during immune responses. Therefore, if the number of infections that a given host acquires falls then the number of regulatory T cells in the repertoire might also fall. The host, if appropriately genetically pre-disposed, is thus in danger of generating autoimmune responses.

Studies published this year have provided evidence that infection can indeed induce the formation of regulatory T cells. McGuirk and colleagues (49) demonstrated the induction of regulatory T cells at a mucosal surface following infection of the murine respiratory tract with *Bordetella pertussis*. Even more interestingly, they describe how the generation of such cells is probably directed by high levels of IL-10 made by DC in response to a pathogen derived molecule FHA (filamentous haemagglutinin). This has interesting parallels with the ability of the *S.mansoni* soluble egg antigen to increase IL-10 production by DC in response to LPS stimulation.

In their experiments McGuirk and colleagues isolated pathogen-specific T cells from the respiratory tracts of mice following infection with *B.pertussis* and cultured them *in vitro*. Some of the clones established that were responsive to restimulation with *B.pertussis* antigens produced high levels of the immunomodulatory cytokine IL-10 and were able to suppress the proliferation and Th1-skewed cytokine production of other T cells specific for *B.pertussis* both *in vitro* and *in vivo*.

In another interesting study, Kullberg *et al.* (50), use an experimental model of colitis to demonstrate the induction of regulatory T cells by infection. It is known that IL-10 knockout mice develop colitis after infection with *Helicobacter hepaticus*. If T cells from these mice are used to re-constitute the immune systems of SCID recipients, these SCID mice also develop colitis. If T cells from normal mice are co-transferred with the T cells from IL-10 deficient mice, colitis still occurs. However, if T cells from normal mice that have previously been infected with *Helicobacter hepaticus* are used to re-constitute these mice, then colitis does not occur.

The implication of this impressive study is that infection with *Helicobacter hepaticus* has generated regulatory T cells in the normal mouse via an IL-10 dependent mechanism. Indeed, treatment of the recipient with anti-IL-10 receptor, which blocks IL-10 signalling abrogates the protective effect of the donor T cells. These animal models are elegant but are conducted in precisely controlled and somewhat artificial animal systems. Do they have any bearing on the mechanisms by which infection may prevent human autoimmune disease? In fact, increasing evidence in human systems also points to a role for IL-10 dependent regulation of inappropriate immune responses. It is interesting to note though, that in the BCG model of diabetes protection described by Serreze, mice that were unable to make IL-10 could be protected by BCG. This might suggest that different infections prevent autoimmune diseases through different mechanisms and while the induction of IL-10-producing regulatory cells may be an important factor in some models, it is not key to BCG-mediated protection.

The notion that infection can induce the formation of regulatory T cells has gained credence with evidence that individuals chronically infected with helminths have low levels of allergy (51). Both allergy and helminth infection cause individuals to make strong Th2 responses and one might expect that if the immune system is primed to making Th2 responses because of chronic helminth infection, such patients might be more susceptible to allergy. In fact, there is a *negative* association between the incidence of helminth infections and allergy. This has been cited as further evidence against the “Th2 skewing” hypothesis of autoimmunity. Instead, it has been proposed that the induction of regulatory T cells by helminth infection prevents allergy and autoimmunity alike.

This idea is attractive for many reasons. Firstly chronic parasitic infections and helminth infections in particular are associated with poor antigen-specific T cell proliferative responses and cytokine production. In the case of schistosomiasis, there is also evidence that patients have elevated circulating levels of IL-10. It remains to be seen

though whether this IL-10 is actually derived from regulatory T cells.

In agreement with this hypothesis, Van der Kleij and colleagues (52) report that human DC that have been treated with phosphatidyl serine derived from *S.mansoni* worms can induce the formation of Th2 cells and regulatory T cells that can suppress the proliferation of other T cells in culture. They also showed that the capacity of the dendritic cell to drive regulatory T cell formation was dependent on ligation of toll-like-receptor 2 on the DC.

T cell Homeostasis as a Mechanism of Protection

While the induction of regulatory T cells by infection may seem a compelling argument, the protection from autoimmunity may occur through an even simpler mechanism. Stockinger *et al.* (53) argue that simply by competing for proliferation and survival signals that might be provided by TCR-MHC interactions and cytokines, T cells of different specificities to autoreactive T cells can prevent the expansion of the damaging autoreactive cells. In her convincing model, Stockinger proposes that T cell regulation can be carried out by any cell that is capable of proliferation in response to stimulation because the proliferating clone will then compete with autoreactive clones. This theory would argue, therefore, that infection does not *need* to induce regulatory T cell populations; “regulation” of a sort occurs simply because the infection drives the expansion of pathogen-specific clones at the expense of autoreactive clones.

Stockinger also argues that much of the evidence for the regulatory T cell hypothesis can also be interpreted as simple homeostatic expansion of transferred cells because the hosts that develop autoimmunity without donor cell intervention are usually congenitally lymphopenic or rendered lymphopenic by irradiation. There is therefore “space” for the transferred T cells to expand into. Stockinger also notes that while “regulatory” T cells are often refractory to proliferation in culture, they can often expand greatly when transferred into lymphopenic hosts.

Despite these arguments, the ability of certain cells to suppress proliferation of their fellow T cells in culture and the immunosuppressive capabilities of T cell-derived cytokines like IL-10 and TGF-beta would suggest that some form of regulatory T cell does exist. Recently, O’Garra and colleagues showed that it was possible to generate a pure IL-10-producing population of T cells *in vitro* using vitamin D3 and dexamethasone further strengthening the evidence that a specialised suppressor cells can form part of a T cell repertoire (54).

AND NOW THE BAD NEWS: VIRUSES MAY TRIGGER AUTOIMMUNITY

It is easy to understand the excitement generated by the ability of infections to halt autoimmune processes. The phenomenon not only suggests simple therapeutic interventions but also offer insights into how the immune system deals with the conflict at the heart of its existence – vigorously attacking pathogens while remaining tolerant of self. There is certainly an elegance to the notion that the key

function of attack might re-enforce the opposite, though equally important requirement for tolerance. It is timely, then to remind ourselves that there is also a substantial body of literature implicating infections in *precipitating* autoimmune diseases. In particular, in both murine and human diabetes, there is evidence to suggest that viruses can act to trigger the disease in genetically susceptible individuals.

Various strains of the enterovirus family have been implicated in type I diabetes but the Coxsackie virus members of this family have come under the closest scrutiny (55). In the human disease, there is epidemiological data that suggests a high frequency of IgM antibodies against Coxsackie B in children that were recently diagnosed with diabetes. Some contradictory reports, however have failed to find differences in antibody titre between diabetic and non-diabetic patients. Coxsackie B4 and B5 strain viruses have been isolated from the pancreas of patients with acute-onset diabetes. Extensive research has also investigated the capability of coxsackie viruses to cause diabetes in NOD mice. Coxsackie B4 viruses and other serotypes can induce diabetes in certain mouse strains if the virus has been passaged in cultured beta cells to shift the tropism of the virus from pancreatic acinar cells to beta cells.

It is not quite clear why these viruses can induce diabetes but several mechanisms that are not mutually exclusive have been proposed. Coxsackie viruses are cytolytic so direct destruction of beta cells might account in part for the loss of glycaemic control. In addition, infection of beta cells with the virus appears to up-regulate the expression of GAD-65, a target antigen in both human and murine disease. This might therefore lead to increased presentation of this antigen to autoreactive T cells. Another likely explanation for disease-triggering might be molecular mimicry, whereby the virus expresses antigens that are sufficiently similar to host antigens that an immune response to viral antigens will also lead to the destruction of host cells.

However, a recent paper (56) argues that inoculation of 4-8 week-old NOD mice with any of nine different Coxsackie B virus strains actually reduced the incidence of diabetes 2-10 fold over a ten month period.

CONCLUSIONS AND FUTURE DIRECTIONS

The evidence that bacterial and parasitic infection can prevent autoimmunity has become compelling in recent years. This is exciting news both for immunologists attempting to discern the complex physiology of the immune system and clinicians attempting to halt the alarming rise in autoimmunity. What is also becoming clear is that multiple mechanisms may account for this protection and may include Th1/Th2 deviation, regulation and homeostatic mechanisms (Fig. 1) It is likely that not all infections may protect by the same mechanisms; indeed one infection may utilise multiple mechanisms to abrogate autoimmune disease.

The success and relative safety of vaccination programmes for infection and the ability of some microbial or even helminth antigens like SWA and SWA to prevent diabetes highlights the potential of “autoimmune vaccination.” This optimism must be tempered, however, by the complex nature of autoimmune disease. There are many

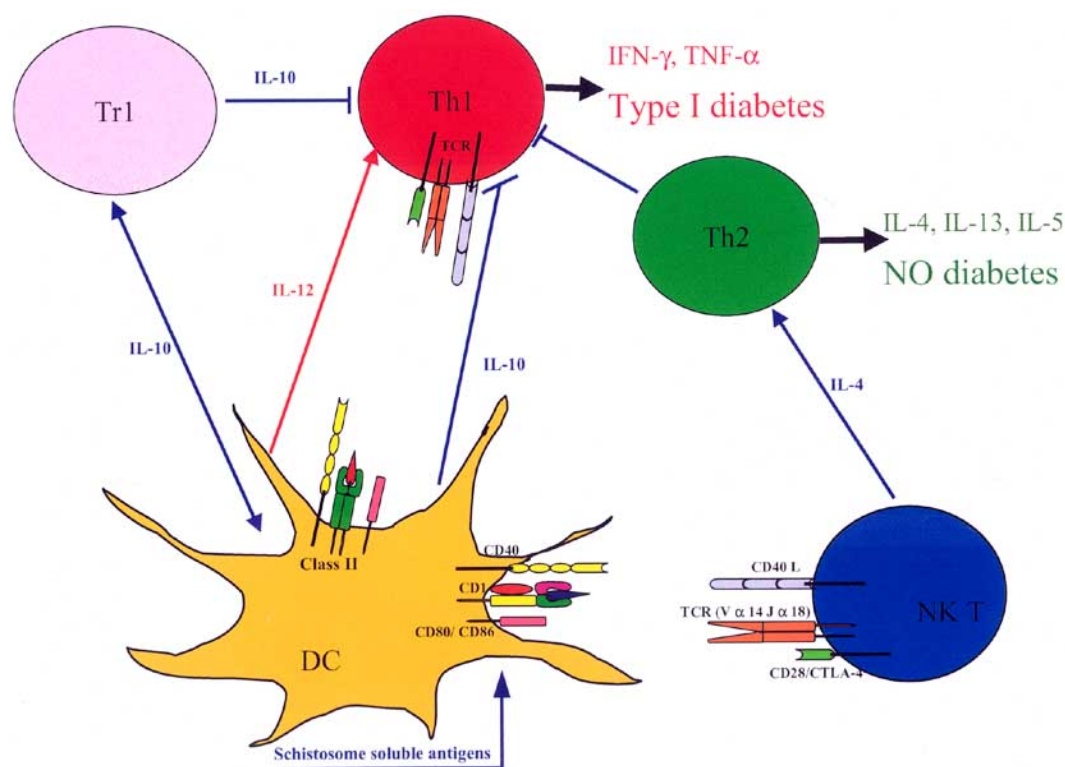


Fig. (1). Diagram summarising the possible mechanisms by which helminth infection may prevent Type I diabetes.

genetic susceptibility factors and these must be explored more thoroughly to truly appreciate the nature of autoimmune disease. It is important to note that individuals with one autoimmune disease are also at higher risk of getting others; therefore it would be imperative to establish that the “vaccination” against autoimmune disease does not merely precipitate another autoimmune syndrome.

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ABBREVIATIONS

NOD mouse	=	Non-Obese Diabetic mouse
NKT cells	=	Natural Killer T cells
CFA	=	Complete Freund's Adjuvant
BCG	=	Bacillus Calmette Guerin
SWA	=	Soluble Worm Antigen
SEA	=	Soluble Egg Antigen
SLE	=	Systemic Lupus Erythematosus
APC's	=	Antigen Presenting Cells
PAMP	=	Pathogen Associated Molecular Pattern
PRR	=	Pattern Recognition Receptors

DC	=	CD1d -/-CD1 knockout
CD	=	Complementarity determining
TGF-β	=	Transforming Growth Factor Beta;
FHA	=	Filamentous Haemagglutinin
GAD	=	Glutamic Acid Decarboxylase

REFERENCES

- [1] Gale, E. A. The rise of childhood type 1 diabetes in the 20th century. *Diabetes* **2002**, *51*, 3353-3361.
- [2] Holgate, S. T. The epidemic of allergy and asthma. *Nature* **1999**, *402*, B2-4.
- [3] Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* **1998**, *351*, 1225-1232.
- [4] Wicker, L. S.; Todd, J. A.; Peterson, L. B. Genetic control of autoimmune diabetes in the NOD mouse. *Annu. Rev. Immunol.* **1995**, *13*, 179-200.
- [5] Vyse, T. J.; Todd, J. A. Genetic analysis of autoimmune disease. *Cell* **1996**, *85*, 311-318.
- [6] Sadelain, M. W.; Qin, H. Y.; Lauzon, J.; Singh, B. Prevention of type I diabetes in NOD mice by adjuvant immunotherapy. *Diabetes* **1990**, *39*, 583-589.
- [7] Harada, M.; Kishimoto, Y.; Makino, S. Prevention of overt diabetes and insulinitis in NOD mice by a single BCG vaccination. *Diabetes Res. Clin. Pract.* **1990**, *8*, 85-89.
- [8] Yagi, H.; Matsumoto, M.; Kishimoto, Y.; Makino, S.; Harada, M. Possible mechanism of the preventive effect of BCG against diabetes mellitus in NOD mouse. II. Suppression of pathogenesis by macrophage transfer from BCG-vaccinated mice. *Cell Immunol.* **1991**, *138*, 142-149.
- [9] Lakey, J. R.; Singh, B.; Warnock, G. L.; Rajotte, R. V. BCG immunotherapy prevents recurrence of diabetes in islet grafts

- transplanted into spontaneously diabetic NOD mice. *Transplantation* **1994**, 57, 1213-1217.
- [10] Lakey, J. R.; Warnock, G. L.; Singh, B.; Rajotte, R. V. Adjuvant immunotherapy with Bacillus Calmette-Guerin prevents recurrence of diabetes in islet grafts transplanted into NOD mice. *Transplant. Proc.* **1994**, 26, 736.
- [11] Cooke, A.; Tonks, P.; Jones, F. M.; O'Shea, H.; Hutchings, P. *et al.* Infection with *Schistosoma mansoni* prevents insulin dependent diabetes mellitus in non-obese diabetic mice. *Parasite Immunol.* **1999**, 21, 169-176.
- [12] Kullberg, M. C.; Pearce, E. J.; Hieny, S. E.; Sher, A.; Berzofsky, J. A. Infection with *Schistosoma mansoni* alters Th1/Th2 cytokine responses to a non-parasite antigen. *J. Immunol.* **1992**, 148, 3264-3270.
- [13] Baxter, A. G.; Horsfall, A. C.; Healey, D.; Ozegbe, P.; Day, S. *et al.* Mycobacteria precipitate an SLE-like syndrome in diabetes-prone NOD mice. *Immunology* **1994**, 83, 227-231.
- [14] Fearon, D. T.; Locksley, R. M. The instructive role of innate immunity in the acquired immune response. *Science* **1996**, 272, 50-53.
- [15] Janeway, C. A., Jr. Approaching the asymptote? Evolution and revolution in immunology. *Cold. Spring. Harb. Symp. Quant. Biol.* **1989**, 54 Pt 1, 1-13.
- [16] Medzhitov, R.; Preston-Hurlburt, P.; Janeway, C. A., Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* **1997**, 388, 394-397.
- [17] Underhill, D. M.; Ozinsky, A. Toll-like receptors: key mediators of microbe detection. *Curr. Opin. Immunol.* **2002**, 14, 103-110.
- [18] Turley, S. J. Dendritic cells: inciting and inhibiting autoimmunity. *Curr. Opin. Immunol.* **2002**, 14, 765-770.
- [19] Feili-Hariri, M.; Falkner, D. H.; Morel, P. A. Regulatory Th2 response induced following adoptive transfer of dendritic cells in prediabetic NOD mice. *Eur. J. Immunol.* **2002**, 32, 2021-2030.
- [20] Steinman, R. M.; Nussenzweig, M. C. Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA* **2002**, 99, 351-358.
- [21] Banchereau, J.; Steinman, R. M. Dendritic cells and the control of immunity. *Nature* **1998**, 392, 245-252.
- [22] Banchereau, J.; Briere, F.; Caux, C.; Davoust, J.; Lebecque, S. *et al.* Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **2000**, 18, 767-811.
- [23] Demangel, C.; Bean, A. G.; Martin, E.; Feng, C. G.; Kamath, A. T. *et al.* Protection against aerosol Mycobacterium tuberculosis infection using Mycobacterium bovis Bacillus Calmette Guerin-infected dendritic cells. *Eur. J. Immunol.* **1999**, 29, 1972-1979.
- [24] MacDonald, A. S.; Pearce, E. J. Cutting edge: polarized Th cell response induction by transferred antigen-pulsed dendritic cells is dependent on IL-4 or IL-12 production by recipient cells. *J. Immunol.* **2002**, 168, 3127-3130.
- [25] MacDonald, A. S.; Straw, A. D.; Dalton, N. M.; Pearce, E. J. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J. Immunol.* **2002**, 168, 537-540.
- [26] Whelan, M.; Harnett, M. M.; Houston, K. M.; Patel, V.; Harnett, W. *et al.* A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J. Immunol.* **2000**, 164, 6453-6460.
- [27] Reis e Sousa, C.; Edwards, A. D.; Manickasingham, S. P.; Schulz, O. Conditioning of dendritic cells by pathogen-derived stimuli. *Immunobiology* **2001**, 204, 595-597.
- [28] Abbas, A. K.; Murphy, K. M.; Sher, A. Functional diversity of helper T lymphocytes. *Nature* **1996**, 383, 787-793.
- [29] Pearce, E. J.; MacDonald, A. S. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* **2002**, 2, 499-511.
- [30] Sharif, S.; Delovitch, T. L. Regulation of immune responses by natural killer T cells. *Arch. Immunol. Ther. Exp. (Warsz)* **2001**, 49 Suppl 1, S23-31.
- [31] Kronenberg, M.; Gapin, L. The unconventional lifestyle of NKT cells. *Nat. Rev. Immunol.* **2002**, 2, 557-568.
- [32] Gombert, J. M.; Herbelin, A.; Tancrede-Bohin, E.; Dy, M.; Carnaud, C. *et al.* Early quantitative and functional deficiency of NK1+/-like thymocytes in the NOD mouse. *Eur. J. Immunol.* **1996**, 26, 2989-2998.
- [33] Baxter, A. G.; Kinder, S. J.; Hammond, K. J.; Scollay, R.; Godfrey, D. I. Association between alphabetaTCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. *Diabetes* **1997**, 46, 572-582.
- [34] Wilson, S. B.; Kent, S. C.; Patton, K. T.; Orban, T.; Jackson, R. A. *et al.* Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. *Nature* **1998**, 391, 177-181.
- [35] Wilson, S. B.; Kent, S. C.; Horton, H. F.; Hill, A. A.; Bollyky, P. L. *et al.* Multiple differences in gene expression in regulatory Valpha 24Jalpha Q T cells from identical twins discordant for type 1 diabetes. *Proc. Natl. Acad. Sci. USA* **2000**, 97, 7411-7416.
- [36] Lehuen, A.; Lantz, O.; Beaudoin, L.; Laloux, V.; Carnaud, C. *et al.* Overexpression of natural killer T cells protects Valpha14-Jalpha281 transgenic nonobese diabetic mice against diabetes. *J. Exp. Med.* **1998**, 188, 1831-1839.
- [37] Sharif, S.; Arreaza, G. A.; Zucker, P.; Mi, Q. S.; Sondhi, J. *et al.* Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat. Med.* **2001**, 7, 1057-1062.
- [38] Hong, S.; Wilson, M. T.; Serizawa, I.; Wu, L.; Singh, N. *et al.* The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat. Med.* **2001**, 7, 1052-1056.
- [39] Gallichan, W. S.; Kafri, T.; Krah, T.; Verma, I. M.; Sarvetnick, N. Lentivirus-mediated transduction of islet grafts with interleukin 4 results in sustained gene expression and protection from insulinitis. *Hum. Gene Ther.* **1998**, 9, 2717-2726.
- [40] Serreze, D. V.; Chapman, H. D.; Post, C. M.; Johnson, E. A.; Suarez-Pinzon, W. L. *et al.* Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. *J. Immunol.* **2001**, 166, 1352-1359.
- [41] Maloy, K. J.; Powrie, F. Regulatory T cells in the control of immune pathology. *Nat. Immunol.* **2001**, 2, 816-822.
- [42] Sakaguchi, S.; Sakaguchi, N.; Asano, M.; Itoh, M.; Toda, M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol.* **1995**, 155, 1151-1164.
- [43] Shevach, E. M.; McHugh, R. S.; Piccirillo, C. A.; Thornton, A. M. Control of T-cell activation by CD4+ CD25+ suppressor T cells. *Immunol. Rev.* **2001**, 182, 58-67.
- [44] Powrie, F.; Leach, M. W.; Mauze, S.; Menon, S.; Caddle, L. B. *et al.* Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* **1994**, 1, 553-562.
- [45] Powrie, F.; Carlino, J.; Leach, M. W.; Mauze, S.; Coffman, R. L. A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. *J. Exp. Med.* **1996**, 183, 2669-2674.
- [46] Groux, H.; O'Garra, A.; Bigler, M.; Rouleau, M.; Antonenko, S. *et al.* A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **1997**, 389, 737-742.
- [47] Fowell, D.; Mason, D. Evidence that the T cell repertoire of normal rats contains cells with the potential to cause diabetes. Characterization of the CD4+ T cell subset that inhibits this autoimmune potential. *J. Exp. Med.* **1993**, 177, 627-636.
- [48] Seddon, B.; Mason, D. Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. *J. Exp. Med.* **1999**, 189, 877-882.
- [49] McGuirk, P.; McCann, C.; Mills, K. H. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. *J. Exp. Med.* **2002**, 195, 221-231.
- [50] Kullberg, M. C.; Jankovic, D.; Gorelick, P. L.; Caspar, P.; Letterio, J. J. *et al.* Bacteria-triggered CD4(+) T regulatory cells suppress *Helicobacter hepaticus*-induced colitis. *J. Exp. Med.* **2002**, 196, 505-515.
- [51] Yazdanbakhsh, M.; Kremsner, P. G.; van Ree, R. Allergy, parasites, and the hygiene hypothesis. *Science* **2002**, 296, 490-494.
- [52] Van Der Kleij, D.; Latz, E.; Brouwers, J. F.; Kruijs, Y. C.; Schmitz, M. *et al.* A novel host - parasite lipid cross talk: schistosomal lyso-phosphatidylserine activates toll-like receptor-2 and affects immune polarization. *J. Biol. Chem.* **2002**
- [53] Stockinger, B.; Barthlott, T.; Kassiotis, G. T cell regulation: a special job or everyone's responsibility? *Nat. Immunol.* **2001**, 2, 757-758.

- [54] Barrat, F. J.; Cua, D. J.; Boonstra, A.; Richards, D. F.; Crain, C. *et al.* *In vitro* generation of interleukin 10-producing regulatory CD4(+) T cells is induced by immunosuppressive drugs and inhibited by T helper type 1 (Th1)- and Th2-inducing cytokines. *J. Exp. Med.* **2002**, 195, 603-616.
- [55] Jaeckel, E.; Manns, M.; Von Herrath, M. Viruses and diabetes. *Ann. N. Y. Acad. Sci.* **2002**, 958, 7-25.

- [56] Tracy, S.; Drescher, K. M.; Chapman, N. M.; Kim, K. S.; Carson, S. D. *et al.* Toward testing the hypothesis that group B coxsackieviruses (CVB) trigger insulin-dependent diabetes: inoculating nonobese diabetic mice with CVB markedly lowers diabetes incidence. *J. Virol.* **2002**, 76, 12097-12111.

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