Infection and autoimmunity: are we winning the war, only to lose the peace?

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It appears that, in developed countries, as we succeed in reducing the incidence of infections we could expose underlying predispositions to develop autoimmunity. This article outlines some of the potential mechanisms that are induced by infection and that prevent onset of autoimmunity, and particularly focuses on the autoimmune disease Type 1 diabetes. Studies on the protective effects of pathogens as diverse as parasitic helminths and intracellular bacteria suggest the possibility that different classes of infectious organism mediate different protective mechanisms. Parasites and the mammalian immune system have evolved together to limit damage to host tissue, which is important to the host for self-maintenance and to the parasite, in some cases, to maintain its habitat and complete its life cycle. By understanding the mechanisms by which different classes of parasites modulate the host immune response, we might be able to devise therapeutic strategies for the treatment of autoreactive responses.

Our complex relationships with infectious agents, including parasites, have been evolving since before humans emerged as a species. The ability to mount the most appropriate defensive responses against pathogens ranging from 20-foot-long intestinal tapeworms to intracellular viruses, bacteria or protozoa, yet causing the least amount of collateral damage to host tissues, will have been a major selective pressure throughout evolutionary time. A balanced, non-self-damaging, but effective response to infectious agents would have been, and still is, an important element in the genetic fitness of the individual.

Infection is still a major environmental threat even in the economically developed and sanitized Western world, as made clear by the effects of acquired or genetic immune deficiencies. However, in the developed world, the majority of people do not die from the effects of infectious diseases. The constant presence of chronically debilitating helminths and zoonotic infections, as well as the daily risk of exposure to food-, water- and vector-borne infection, which is still widespread in developing countries, was largely controlled during the course of the 20th century. This relatively aseptic life represents a striking departure from the environmental experience that moulded our immune system. Differences in immune responses have been described between African and European human populations, and it is thought possible that these are due to the different levels of exposure to infectious organisms throughout life [1]. Nowadays, many of the diseases of the developed world are non-communicable conditions associated with old age or recent affluence-driven lifestyle changes (the ability to make choices for the good of our own future health is probably a recent burden in evolutionary time). However, the developed countries are now also suffering a well-documented and alarming increase in the incidence of a whole range of ‘immunological diseases’ categorized around allergy or autoimmunity.

The onset of these diseases is undoubtedly influenced by both genetic and environmental factors. For example, autoimmune diseases such as Type 1 diabetes and systemic lupus erythematosus (SLE) are increasing in incidence in the developed world and have concordance rates for development in identical twins of around 40% [2]. The dramatic recent increase in diabetes suggests a role for an environmental effect. A common external aetiological agent for Type 1 diabetes, such as viral infection, has not been established. In fact, the increase in the incidence of a whole range of autoimmune diseases, plus the relative absence of autoimmunity in developing countries where chronic infectious diseases are endemic, has suggested an alternative hypothesis. That is, infection might actually inhibit the development of autoimmunity, such that the epidemic of autoimmune disease in the developed world is a consequence of the sudden decline in the experience of infection that has recently occurred in these human populations [3].

The ‘hygiene hypothesis’

Experiments carried out using animal models of human autoimmune diseases have shown that infections can prevent the onset of autoimmunity (Table 1). These findings, together with similar results showing that infection might also protect against asthma, have led to the so-called ‘hygiene hypothesis’ in which it is envisaged that the control of infection through increased cleanliness and antibiotics predisposes to the development of such diseases [4,5]. The induction of regulatory cytokines by a wide range of infectious organisms has been suggested as a common mechanism by which a high level of exposure to different pathogens would be protective against T helper 2...
is increasing at 2.5% per year in 0–14-year-old children. Africa has the lowest number of cases, with slightly North America with number, with 1.6 million Type 1 diabetics, followed by Europe contains the largest live with Type 1 diabetes, and more than 218 000 people secrete insulin. More than 5.3 million people worldwide the immune system destroy the pancreatic

Table 1. Infection inhibits autoimmunity

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<th>Infection</th>
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*(P. Zaccone, unpublished.)*

(Th2)-cell hypersensitivities [6] and T helper 1 (Th1)- or Th2-mediated autoimmune diseases. However, the ways in which infection might inhibit the development of autoimmunity and asthma remain to be fully clarified. Using Type 1 diabetes as a model autoimmune disease, some of the potential mechanisms by which autoimmune diseases may be prevented by infection will be discussed.

What is Type 1 diabetes?

Type 1 diabetes is an autoimmune disease in which cells of the immune system destroy the pancreatic β cells that secrete insulin. More than 5.3 million people worldwide live with Type 1 diabetes, and more than 218 000 people per year develop the disease. Europe contains the largest number, with 1.6 million Type 1 diabetics, followed by North America with ~1 million affected individuals. Africa has the lowest number of cases, with slightly more than 100 000. The incidence of this disease in the UK is increasing at 2.5% per year in 0–14-year-old children.

The development of Type 1 diabetes both in humans and spontaneous rodent models is under the control of many genes [7]. Since the disease is of juvenile onset and was considered fatal before the discovery of insulin by Banting and Best in 1922 [8], it might be expected that individuals carrying the genes predisposing to this disorder would not be represented in the current population. The fact that this is not the case suggests that either the genes predisposing to diabetes or genes in linkage disequilibrium have been retained because they convey a selective advantage. As both mycobacterial and helminth infections were endemic in northern Europe until relatively recently in evolutionary time, these organisms clearly could have played a role in such selection. The association of resistance to mycobacteria with a predisposition to develop the autoimmune diseases SLE and haemolytic anaemia in an animal model provides support for this hypothesis [9].

Studies in a mouse model of Type 1 diabetes, the non-obese diabetic (NOD) mouse (Box 1), have shown that some infections prevent onset of diabetes. This effect of infection is not unique to Type 1 diabetes, and can be seen in several other spontaneous or experimentally induced autoimmune conditions (Table 1).

How do infections prevent onset of Type 1 diabetes?

To understand how infections might modify onset of Type 1 diabetes, it is useful to examine the cell types that have been shown to be involved in the development of this autoimmune disease. It is thought that priming of Th1 cells and CD8+ T cells specific for islet antigen occurs in the pancreatic draining lymph node (Figure 1a). These cells migrate to the pancreatic islets and recruit other cell types including macrophages. Pancreatic β cells might be destroyed in a variety of ways, including (i) major histocompatibility complex (MHC) class I-restricted killing by cytotoxic CD8+ T cells, (ii) following cytokine or free radical release by macrophages and T cells [10], and (iii) death induced by a Fas–Fas ligand (FasL) interaction between T cells and the β cell (Figure 1b). This pathogenic response to β cells can be modulated by natural killer T (NKT) cells (Box 2) and T regulatory (Treg) cells (Box 3). NOD mice are deficient in NKT cells [11] and any manipulation that boosts their numbers and activates these cells, such as injection of α-galactosylceramide, prevents diabetes [12,13]. In addition

Box 1. The NOD mouse

The NOD mouse was originally isolated in Japan in the late 1970s by Makino [19] and, together with the BB rat, is one of the most studied models of autoimmune Type 1 diabetes. As in the human disease, diabetes in the NOD mouse is under polygenic control and could be modified by environmental factors. The environmental conditions (temperature [28] and hygiene) in which the animals are kept are very important for maintaining a high incidence of diabetes in the colony. The pancreas of the NOD mouse is gradually infiltrated by macrophages, B cells and T cells (CD4+ and CD8+ T cells) and, eventually, the β-cell mass is destroyed. Dendritic cells play a key role in the initiation of the autoimmune process by presenting autoantigens, with the consequent generation of autoreactive T cells. The incidence of diabetes is variable between different NOD mouse colonies, and female NOD mice normally develop diabetes with a higher incidence than male mice. Both female and male mice start to develop a pancreatic infiltrate around 6 weeks of age. At this stage, the infiltrate is mainly localized around the islet and the pancreatic ducts. Between 8 and 12 weeks, the infiltrate moves into the islet, β-cell destruction occurs and clinical signs of disease start to appear. NOD mice develop glycaemia and glycosuria and, by the age of 30 weeks, 70–80% of the female mice are diabetic. In many colonies, only ~20% of the males ever develop clinical signs of disease.

Box 2. NKT cells and autoimmune diseases

Natural killer T (NKT) cells are a lymphocyte subpopulation with important regulatory functions in fighting infection and in autoimmune diseases [36]. NKT cells can promptly secrete interferon (IFN)-γ or interleukin (IL)-4, depending on the nature of the pathogen, creating a very important bridge between the innate and the adaptive immune system. This heterogeneous cell type co-expresses NKT and T-cell receptors. In the mouse, for instance, NKT cells have been divided into at least four categories, based upon expression of different NKT and T-cell receptors and co-receptors, antigen-presenting molecules and location within different organs of the immune system. Of particular interest in the context of autoimmune diseases is the Vα14-Jα18 TCR-positive, CD1d-restricted subset of NKT cells. Defects in this distinct NKT-cell type have been implicated in numerous T helper 1 (Th1)-mediated autoimmune diseases, including diabetes, multiple sclerosis, rheumatoid arthritis, lupus and systemic sclerosis. Glycolipids, particularly α-galactosylceramide, presented by CD1d molecules induce proliferation and activation of Vα14-Jα18 TCR-positive NKT cells [36].
to a deficiency in NKT cells, there has been some suggestion of defective Treg function in NOD mice [14].

**Does schistosomiasis prevent diabetes by skewing the Th1–Th2 balance?**

Infection of NOD mice with *Schistosoma mansoni* prevents onset of Type 1 diabetes. As Type 1 diabetes is a Th1-mediated disease, the ability of *S. mansoni* to prevent diabetes onset could be attributed to a skewing of the immune response towards a Th2 response. It is well known that infection with *S. mansoni* can have profound effects on immune responses to non-parasite antigens as well as on those to other infections. For example, T-cell responses to sperm whale myoglobin were skewed towards a Th2-type cytokine production in *S. mansoni*-infected mice [15]. In addition, co-infection studies have confirmed wide-ranging effects of infection with *S. mansoni*. Infection with *S. mansoni* affects the course of both *Leishmania major* and *Trichuris muris* infections [16,17]. With regard to the response to *Trichuris muris*, co-infection with *S. mansoni* permitted a susceptible mouse strain to mount an effective Th2 response and clear *Trichuris*. In the case of *L. major* infection, there was a delayed resolution of lesion onset that might have been owing to impaired macrophage killing of the intracellular organism. After infection, the response to *L. major* was skewed by a Th2-type response.
NKT cells in the context of CD1d (Box 2), this suggests that infection has somehow altered DC development, their differential survival or has influenced their trafficking, any of which could influence the context of autoantigen presentation (Box 4). However, infection with Salmonella does not result in the loss or permanent inactivation of T cells able to cause diabetes. These cells can be shown to be present in the spleens of nondiabetic NOD mice 10 months after Salmonella infection. This is in marked contrast to the situation in NOD mice protected from diabetes onset by exposure to schistosome antigens, where diabetogenic T cells appear to be ineffective. Differential trafficking of T cells as a result of bacterial infection might play a part in controlling the development of Type 1 diabetes. It is thought that T cells in NOD mice become primed to islet antigen in the pancreatic draining lymph node and then traffic to the pancreas to mediate β-cell destruction [26]. Analogously, redirected trafficking of central nervous system (CNS)-specific T cells following mycobacterial infection has been shown to provide protection against experimentally induced allergic encephalomyelitis [27].

Concluding remarks

Studies in animal models of human autoimmune disease strongly suggest that some infections are able to prevent development of autoimmune pathology. We have shown that the mechanisms giving rise to this protection vary with different infections. This could stem from the

Box 3. T regulatory cells

The existence of a suppressor CD4+ T-cell population capable of containing the effect of T effector cells was postulated many years ago [45]. These cells are now referred to as T regulatory (Treg) cells. In the past five years, many groups have contributed to achieving an understanding of Treg cells and their role in controlling autoimmunity, infections and tumour growth. The most recent classification of this important T-cell population divides it into two main subclasses: naturally occurring Treg cells and induced Treg cells (or Tr1). Naturally occurring Treg cells are CD4+ T cells that mature in the thymus and constitutively express CD25. They can also express GITR (TNFRSF18), CTLA-4, CD103 and CD134. Unfortunately, none of these markers is exclusive for Treg cells. Recent studies have identified expression of the Foxp3 transcription factor as a unique attribute of naturally occurring Treg cells. Induced Treg cells are T cells that are generated from CD4+CD25- thymocytes and acquire a variable expression of CD25 in the periphery. Induced Treg cells can also express the other surface markers described above but there is no evidence that they can express Foxp3. Both subtypes can mediate suppression mediated by cytokine secretion [interleukin (IL)-10 and/or transforming growth factor (TGF)-β]. However, in vivo suppression by both Treg subtypes is apparently cytokine mediated (IL-4, IL-10 and/or TGF-β) [46].

at four weeks to a Th2 response with a downregulation of specific interferon (IFN)-γ, tumour necrosis factor (TNF)-α and nitric oxide (NO) production. However, it is interesting to note that the Th1 response to L. major re-establishes itself, whereas the diabetes-prevention effects of S. mansoni infection are long-lasting. This might suggest that additional mechanisms other than Th1/Th2 skewing play a role in controlling onset of Type 1 diabetes in NOD mice.

A role for the innate immune system: NKT cells and dendritic cells?

Type 1 diabetes can also be prevented in NOD mice by injection of whole eggs or soluble antigens from the schistosome egg (SEA) or the worm (SWA) [18]. However, this is only effective if the injections are started when the mice are 4 weeks of age. The ability of T cells to transfer diabetes is markedly reduced following exposure of NOD mice to schistosome antigens. This could suggest that Treg cells have been induced.

The schistosome products are not therapeutically effective if injections are delayed until later time points when a lymphocytic infiltration has already entered the pancreas [19]. The need for an early exposure suggests that the schistosome antigens are playing a role during disease initiation. Analysis of the effects of SEA and SWA on bone-marrow-derived dendritic cells (DCs) and on NKT cells have shown that SEA induces increased production of interleukin (IL)-10 from DCs together with a corresponding reduction in production of IL-12. In addition, S. mansoni synthesizes glycosphingolipids including galactosylceramide [20]. As glycolipids are presented to NKT cells in the context of CD1d (Box 2), this suggests that S. mansoni antigens might be capable of expanding and activating NKT cells. NKT-cell numbers were significantly enhanced in vivo by injection of SWA and, to a lesser extent, SEA [18]. Thus, it can be envisaged that infection with S. mansoni and exposure to antigens derived from both worm and egg can influence diabetes onset at many levels through the production of anti-inflammatory cytokines (Figure 1c and d). The life cycle of the parasite within the host impacts dramatically on the genetically programmed onset of Type 1 diabetes.

What about other infections?

A case has been made for diabetes prevention through a skewing of the immune response in NOD mice to a Th2 response and production of anti-inflammatory cytokines. However, an immune deviation to a Th2 response is unlikely to explain how bacteria such as Salmonella or Mycobacterium spp. prevent autoimmunity as infection with these agents elicits production of pro-inflammatory cytokines. NOD mice carry the resistant allele of NRampl and, possibly because activated DCs and macrophages from NOD mice make large amounts of IL-12, TNF-α and NO, they are excellent at killing intracellular organisms such as Salmonella [21] and Leishmania [22].

Diabetes can be prevented by bacteria even if the infection is given as late as 12 weeks when pancreatic infiltration is advanced. Studies of the effects of mycobacteria suggest that inhibitory macrophages [23] or Treg cells [24,25] are involved in diabetes prevention. Examination of the effects of Salmonella infection on diabetogenic T cells, DCs and NKT cells in vivo shows a very different picture from that observed with S. mansoni infection. In contrast to the observations following helminth infection, there is a loss of NKT cells following S. typhimurium infection coupled with a marked reduction in the numbers of CD8a+CD11c+ DCs in the spleens and pancreatic draining lymph nodes of infected mice. The observed decrease in the ratio of CD8a+CD11c+ DCs suggests that infection has somehow altered DC development, their differential survival or has influenced their trafficking, any of which could influence the context of autoantigen presentation (Box 4). However, infection with Salmonella does not result in the loss or permanent inactivation of T cells able to cause diabetes. These cells can be shown to be present in the spleens of nondiabetic NOD mice 10 months after Salmonella infection. This is in marked contrast to the situation in NOD mice protected from diabetes onset by exposure to schistosome antigens, where diabetogenic T cells appear to be ineffective. Differential trafficking of T cells as a result of bacterial infection might play a part in controlling the development of Type 1 diabetes. It is thought that T cells in NOD mice become primed to islet antigen in the pancreatic draining lymph node and then traffic to the pancreas to mediate β-cell destruction [26].

Concluding remarks

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Box 4. Dendritic cells, infection and autoimmunity

As the ‘sentinels of the immune system’, dendritic cells (DCs) have a key role to play in the initiation of immune responses to pathogens. Distributed throughout the body, in particular in the skin and mucosae, they are constantly sampling antigens for presentation to T cells. DCs have been divided into several subsets based upon surface marker expression, but the precise functional significance and interrelations of many of these subsets are poorly defined [37]. Nevertheless, it seems that expression of the CD8α+ subset is a useful marker of DC function and has been correlated to several differences in antigen-presenting behaviour. These include the description of roles in the induction of peripheral T-cell tolerance, in which the CD8α+ subset has been characterized as playing a pivotal role involving the presentation of apoptotic ‘self’-material [38]. The proposed mechanisms for such effects include the generation of reactive nitric oxide (NO) [39] and highly localized effects upon tryptophan metabolism [40]. In Salmonella typhimurium infection, pathogens can be visualized within DCs as early as 4 h after infection [41] and are involved in the production of inflammatory cytokines, including tumour necrosis factor (TNF)-α [42]. Studies of Salmonella infection have revealed the induction of apoptosis within infected cells [43], a differential involvement of the CD8α+ DC subset [42; A. Cooke et al., unpublished], and the generation of high levels of cytokines such as interferon (IFN)-γ, which is known to affect tryptophan metabolism [44]. As such, it is interesting to speculate that pathogen-induced modulation of the behaviour of certain DC subsets might be relevant to the observed reduction in autoimmunity.

different requirements of infectious agents to influence the host immune response to maximize their chances of transmission. In the developed world, this intricate balance between host and parasite has been perturbed by our successful war against many erstwhile common infections and this could be, at least in part, responsible for the observed increase in autoimmune disease.

There are many checks and balances built into the immune system to prevent the development of autoimmunity. Individuals prone to develop autoimmune disease might have selective deficits in some of these controlling processes that are restored by infection. There is a growing awareness of the role that certain microorganisms might play in immune homeostasis and the induction of Treg cells through interactions with the innate immune system. Defects in homeostatic mechanisms might result in imbalances within the T-cell functional repertoire, predisposing to inflammation and the development of Type 1 diabetes. Restoration of regulatory mechanisms might be the way in which infection ameliorates autoimmune disease in NOD mice. It is tempting to conclude that a decrease in human infection through generally improved public health and increased use of antibiotics and vaccines might provide an explanation for the rise in autoimmunity in the developed world. To whatever degree this hypothesis proves to be true, detailed study of the ways in which disparate infections influence the onset of autoimmune diseases should result in the development of new therapeutic strategies.

Acknowledgements

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The science publishers, Blackwell, Elsevier, the Harcourt Worldwide STM group, Wolters Kluwer International Health and Science, Springer-Verlag and John Wiley, were approached by the WHO and the British Medical Journal in 2001. Initially, more than 1000 journals will be available for free or at significantly reduced prices to universities, medical schools, research and public institutions in developing countries. The second stage involves extending this initiative to institutions in other countries.

Gro Harlem Brundtland, director-general for the WHO, said that this initiative was ‘perhaps the biggest step ever taken towards reducing the health information gap between rich and poor countries’.

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