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For debate

Autoimmune diabetes and the germ theory of disease

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The terms autoaggression and autoimmune attack reflect a long held view that autoimmune disease is caused by a dysregulated immune system which inappropriately attacks healthy tissue [1]. There is evidence, however, for an alternative, indeed opposite, view – that autoimmunity represents the response to a primary lesion in the target tissue and that autoimmunity, like alloimmunity, is physiological, appropriate and protective [2]. The tissue damage and cell death which result are characteristic of an inflammatory response programmed to eliminate immunogen, remove detritus and isolate the lesion.

Inflammation was recognised at least three and a half thousand years ago, and was long thought to be the cause of disease. The reasoning seemed so logical that it endured to the beginning of the nineteenth century: inflammation caused distress and its resolution led to cure. However, we now accept the opposite, that inflammation is a physiological, appropriate and above all protective *response* to disease. John Hunter was among the first to perceive that inflammation has a secondary rather than primary role. In *A Treatise on the Blood, Inflammation and Gunshot Wounds* [3], Hunter wrote that "inflammation in itself is not to be considered as a disease, but as a salutary operation consequent either to some violence or some disease". The wide application of light microscopy during the nineteenth century, and the demonstration that inflammation was caused by microbes, firmly established the relationship between the microbe as pathogen and the inflammation as protective response. The modern view is enshrined in Pasteur's *Germ Theory of Disease* [4]. It is nevertheless important to recognise that the destruction of healthy tissue associated with infection is mediated largely by the inflammatory response, rather than its cause [5].

Autoimmunity is a form of inflammation. However, the absence of a demonstrable pathogen led to the early assumption and subsequent acceptance that immune autoreactivity must be the cause of disease, rather than a "salutary operation". It is a recently acquired understanding of new modes of infection, gained largely through molecular technology [6], that is beginning to reverse the "pre-Hunterian" view that autoimmune disease is caused by a disorder of the immune system. The insulitis associated with Type 1 (insulin-dependent) diabetes mellitus is a widely studied model of autoimmunity, and much has been learned from attempts to provoke insulitis in transgenic animals where the transgene is linked to the insulin regulatory gene.

In a now classic experiment reported in 1987 [7], Hanahan and colleagues studied the immune response in transgenic mice to the Simian virus 40 (SV40) large T antigen which was expressed on the surface of pancreatic islet Beta cells either early or later during life, according to the lineage of the animal [7]. Those mice expressing SV40T during embryogenesis or the neonatal period proved (predictably) tolerant to the antigen, whereas those in which the expression of antigen was delayed beyond the neonatal period developed antibodies to SV40T and histological evidence of insulitis with infiltrating lymphocytes and Beta-cell damage. The inflammatory reaction did not occur spontaneously but only when, and if, the animal expressed T antigen. The inflammation, not merely the expression of T antigen, appeared to be responsible for the Beta-cell damage.

Inflammatory cell damage can be mediated by the cytokines released from activated monocytes/lymphocytes, or by antibodies. Both may be important to the cell damage which occurs in autoimmunity. Nerup has shown that Beta cells are particularly susceptible to damage by interleukin 1 which is released principally by activated macrophages [8]. Notkins and colleagues transfected rat insulinoma cells with the gene encoding the glycoprotein D of herpes simplex virus type 1 [9]. Transfection did not ad-

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versely affect insulin production *per se*, but when the cells were exposed to glycoprotein D, antibodies and complement, insulin production ceased and the cells were lysed. Untransfected cells were not susceptible to lysis by the combination of anti-herpes simplex virus-1 antibody and complement.

The entire natural history of Beta-cell autoimmunity – from expression of neo-antigen, through insulitis to hyperglycaemia and diabetes – was demonstrated by Roman and her group in transgenic mice expressing an influenza virus haemagglutinin on the surface of their islet Beta cells [10]. Hyperglycaemia developed in mice derived from all founder animals at a frequency varying from 13% to 27%, and was associated with lymphocytic infiltration of the islets and an antibody response to Beta-cell antigens, as well as to the viral haemagglutinin. The development of clinical diabetes was strain-dependent.

While these experiments clearly demonstrate the responsiveness of a normal immune system to the selective expression of neo-antigen, and equally selective destruction of the host cell, they do not address the question of the breakdown in tolerance to *self* antigens which characterises autoimmunity.

An explanation for the loss of tolerance to self antigens in autoimmunity may come from the work of Sarvetnick who induced insulitis and diabetes in transgenic mice in which expression of the lymphokine interferon-γ (IFN-γ) was directed by the insulin promoter [11]. Islet Beta-cell destruction was mediated by lymphocytes, and the islet expression of IFN-γ was alone sufficient to result in the loss of immunological tolerance to normal islet antigens. There is good evidence that to become activated (i.e. to lose tolerance), both B and T lymphocytes require two signals [12]. In the case of the T lymphocytes, the first signal is the antigen/MHC class II complex, and the second a single cytokine or cytokine cocktail, depending on the circumstances. IFN-γ is a normal constituent of inflammatory reactions and is an inducer of co-stimulators necessary for lymphocyte activation; it appears that otherwise unreactive lymphocytes specific for islet antigens were activated directly or indirectly by IFN-γ in these mice.

Taken together, the observations summarised here are consistent with a sequence of events which starts, some time after the tolerising period around birth, with the expression on islet Beta cells of viral neoantigens encoded within the genome of the individual. The trigger is speculative, but could be an environmental, possibly dietary, factor responsible for upregulating insulin promoter activity. Expression of the neoantigen provokes a conventional immune response which is destined to eliminate the antigen, remove the detritus and isolate the lesion. The inflammation leads to Beta-cell damage and, if sustained by the persistence of neoantigen, diabetes. IFN-γ, and perhaps other cytokines, released as non-specific co-stimulators by the inflammation, lead to the activation of lymphocytes complementary to local self antigens and to the generation of organ-specific autoantibodies.

Whether the antibodies are interpreted as evidence for dysregulated immunity, as the result of molecular mimicry or as part of an inflammatory response to neo-antigen expressed on the target cell (the primary lesion), depends critically upon a knowledge of their repertoire. An antibody repertoire, however, is revealed only to the extent of the antigens available in the laboratory to probe it. Healthy human pancreas from renal transplant donors, and soluble islet proteins such as insulin, have long been used to detect islet-related antibodies in patients with Type 1 diabetes, but neither preparation is likely to contain the putative neo-antigen responsible for initiating the insulitis. As a result, neither preparation will detect antibodies to such an antigen in the serum of diabetic subjects, so that its presence will go undetected when sought by conventional means.

Pre-Hunterian medicine looked upon inflammation as the cause of disease, because it neither detected nor suspected the microbes responsible. It may be that autoimmunity has been misinterpreted for a similar reason, that the inflammation in autoimmunity is the response to a primary lesion rather than its cause, and that the autoantibodies to self antigens which characterise autoimmune diseases are merely part of the "salutary operation". The germ theory of autoimmunity has been convincingly demonstrated in transgenic models, but what is the evidence for this mechanism in nature?

Immunopathologically-mediated tissue injury is well recognised in a number of natural infections. Some of these infections may be persistent, whereby a non-cytopathic virus is incompletely cleared and maintains an equilibrium with the immune system over long periods of time, resulting in slow destruction of the host tissue [13]. While it has so far proved difficult to demonstrate virus in the islets of newly-diagnosed human Type 1 diabetes (predictably, perhaps, if insulin deficiency signals the last stages of antigen clearance by a conventional immune response), virus persistence in pancreatic islets has been associated with early hyperglycaemia in mice [14]. Viral particles and/or the RNA/DNA encoding them have also been demonstrated in tissues affected by ongoing collagen diseases, myocarditis, and diseases of the central nervous system, endocrine system and digestive systems [15]. In murine Coxsackie B myocarditis, for example, myocytes expressing neo-antigen can be demonstrated in addition to myocyte-directed cytotoxic T lymphocytes and antibodies against both the neo-antigen and cardiac myosin [16].

An experiment to show that the target tissue, rather than the immune system, was the cause of spontaneous autoimmune insulitis would involve the passive transfer of islets to syngeneic recipients [2], rather than the transfer of donor spleen cells. Yoon and colleagues showed that islets from non-diabetic neonatal BB rats, transplanted into acute diabetic adult BB rats, remained undamaged, while islets taken from adult animals, which had been treated with silica to prevent insulitis, were rapidly destroyed [17]. The observation is consistent with the presence on adult islets of antigens which were not expressed on neonatal islets, and satisfies the modified set of postulates for autoimmunity which were recently proposed to replace those of Milgrom and Witebsky [2, 18].

The three principal hypotheses to explain autoimmunity are immune dysregulation [1], molecular mimicry [19] and the primary lesion theory [2]. At this point in time

it is not clear which is correct, and all three may contribute. Support for the primary lesion theory has moved from the speculative to the demonstrable and, in some instances at least, the lesion may be caused by persistent virus to which the host is immunologically intolerant. Persistent viral infection and chronic inflammation may be analogous to bacterial infection and acute inflammation. Both types of inflammation might properly be viewed as protective, although functional tissue is destroyed in the process.

If the role of autoimmunity is to clear neo-antigen, current attempts with immunosuppression to halt the process for clinical benefit in the short term [20] may encourage viral persistence, with a risk of virus-induced neoplasia in the long term. Those transgenic mice tolerant to the SV40T in Hanahan's original experiments did not live to maturity [7]. They developed insulinomas, rather than insulitis. In humans, chronic active hepatitis, which follows the incomplete clearance of viral hepatitis B infection, is often associated with liver-directed autoantibodies and is complicated in the long term by a high frequency of hepatic carcinoma [21]. Given this perspective, it may be more logical to research strategies which intensify viral clearance than those destined to encourage viral persistence.

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