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Breakdown of T-cell ignorance: The tolerance failure responsible for mainstream autoimmune diseases?

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ABSTRACT

This article explores the possibility that the major autoimmune diseases come about because of the breakdown of T lymphocyte ignorance – that state in which antigen and lymphocyte have never come together in such a way as to induce tolerance or an immune response. By use of transgenic technique to place a foreign antigen/peptide in various mouse tissues the widespread occurrence of ignorance has been observed and information obtained on when it is likely to occur. Now, with the advent of tetramer technique to enrich specific T cells and the recognition of lymphocyte markers indicating whether or not antigen interaction has taken place, ignorance of genuine self-antigens is being examined in mouse and man. In the absence of thymic deletion it seems that tolerance to self-antigens is brought about either by T cell ignorance or T cell regulatory control. The initiating factor in these major diseases is likely to be a change in the condition of the antigen leading to tolerance failure. There is evidence that it is ignorance that breaks down in Type 1 diabetes and systemic lupus erythematosus. If this proves a general rule, it may be because ignorance is the tolerance mechanism most vulnerable to subversion.

1. Introduction

Major autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, type 1 diabetes and multiple sclerosis are not generally present in the earliest years of life, often show a relentless onward march and bring about complex damaging effects while remaining largely independent of each other. One characteristic such 'mainstream' diseases do share is an association with various HLA class II proteins, implying the importance in their development of T cell responses against relevant autoantigens. The tendency has been, however, to emphasise differences in aetiology as opposed to looking for common threads [1]. This article considers the possibility that all these mainstream autoimmune diseases derive from the same failure in the way that T lymphocyte tolerance is maintained, a failure that hitherto has not been in the forefront of explanations for their appearance [2].

T cell tolerance may be divided into two categories – central and peripheral. Central tolerance (CT) refers to the process in the thymus leading to deletion of self-reactive T cells. Peripheral tolerance (PT) consists of three arms – the inhibitory actions of regulatory T cells (Treg) on the activation and activity of T cells, the induction of anergy in T cells through antigen recognition in the absence of co-stimulation and finally the presence of immunological ignorance. T cell ignorance is the state in which T cells do not appear to notice or be affected in any way by the relevant autoantigens [2]. Because of the late onset of the diseases some change in the tolerance arrangements has clearly occurred to make possible the development of the disease.

CT comes about through the presence of self-proteins in the thymus principally under the influence of the AIRE gene [3]. The AIRE process includes tissue-specific proteins, though only 85% of the total coding genome is transcribed in that way and not all this will appear as protein [4]. There is no doubt that breakdown of central tolerance can lead to autoimmune disease. This is shown by mutations in the AIRE gene that lead to combinations of several autoimmune conditions appearing at birth or shortly afterwards [3]. Although there may be, for example, symptoms of diabetes, the combination of disorders and the early onset contrast strikingly with the mainstream disorders. Moreover, the latter do not show a strong association with AIRE mutations [5]. Because of the independence of the mainstream diseases, for a failure in CT to be responsible for their onset one would need to assume that at a certain point in life a problem arose in the thymus with the deletion of T cells with a particular specificity or group of specificities. That this could occur seems unlikely.

There is good reason therefore in turning our attention to PT. In animal models in which Tregs have been rendered inoperative a multitude

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of autoimmune phenomena arise [6]. However, for reasons analogous to those given above in relation to CT, a change in Treg cells specific for a narrow group of epitopes is unlikely to be the initiating factor in the mainstream diseases. This does not mean that Tregs may not have vital roles in preventing autoimmune disease.

A failure of ignorance, anergy or regulation would presumably be consequent on an alteration in the availability of the antigen. Such a change in antigen could come about by infection, inflammation, physical damage or altered metabolic process and could involve changes in the expression level of cognate MHC proteins. Mimicry [7] and post-synthetic modification [8] could also play a part. Antigen would of course give the disease process its specificity.

In fact the concept of ignorance derived from the existence of rare diseases in which physical damage to an organ results in changed antigen availability and an autoimmune state. In the example of sympathetic ophthalmia physical damage to one eye leads to an autoimmune attack on the other [9]. It seems clear that there is a state of ignorance to certain eye protein(s) that is overturned as a result of the injury and that activated T lymphocytes can then find and damage the healthy eye.

In 2003 one of us (M.R.S.) put forward the idea that the mainstream autoimmune diseases come about because of the breakdown of ignorance followed subsequently by the failure of Treg to step into the breach [2]. This idea was intended to apply to any of these diseases whether organ-specific or systemic, and whether the resulting T cell response was driven by Class I or II MHC-peptide in secondary lymphoid tissue. The outcome would thus be the appearance of T cells involved in IgG autoantibody production and/or in inflammatory and cytotoxic responses.

While one cannot exclude the possibility that it is anergy rather than ignorance that breaks down it would seem unsatisfactory that there is a necessity throughout life of having to continuously anergise T cells in order to remain healthy. Moreover, as will be seen, anergy does not feature strongly in the evidence to date. A state of ignorance seems a more practical solution where it can be achieved. In this article the main focus is on the possible failure of ignorance - the transformation of ignorance into undesirable knowledge.

2. The transgenic era

As described earlier [2], the advent of transgenic (TG) mice brought confirmation of the phenomenon of ignorance. A foreign antigen can be inserted into the genome of mice so as to be expressed in a particular tissue, the antigen becoming in effect a self-antigen. Would-be effector T cells specific for this antigen are found to be either deleted, regulated, anergic or ignorant. Sometimes it may be useful to insert a T cell receptor (TCR) transgene either alone [10] or together with the appropriate antigen transgene [11,12].

Key transgenic experiments pointing to the importance of ignorance in preventing autoimmune disease were described in 1991 [13,14]. A viral antigen was expressed in pancreatic islets without causing any apparent immune reaction, but later infection with the appropriate virus led to an immune response and development of autoimmune diabetes. Transgenic experiments carried out since that time have demonstrated the widespread occurrence of ignorance and taught some basic principles as to the conditions under which it may arise. In addition to pancreatic islets, ignorance has been demonstrated transgenically in myelin [10], skeletal muscle [11], melanocytes [12] and a B cell lymphoma [15]. Mostly these studies involved CD4 T cells but ignorant CD8 cells feature strongly in human studies (see below). As might be expected ignorance is favoured by low antigen expression [16], low affinity between TCR and MHC/peptide [11,12] and low affinity between peptide and MHC [10]. Ignorance is more likely to occur where there is no thymic expression of the antigen since that may well result in deletion [17].

A major consideration in such experiments is the nature of the evidence for ignorance. Apart from the absence of lymphocyte proliferation and/or a specific immune response there will be the demonstration that an immune response does occur following injection of the antigen concerned, or appropriate infection [12,13]. This will exclude clonal deletion and may argue against T cell anergy or regulation [18]. The evidence for ignorance is greatly strengthened by showing that T lymphocytes sensitive to the peptide concerned remain in a naïve state with non-appearance of lymphocyte activation markers or markers associated with anergy [19]. CD44 may prove to be the ideal marker for this purpose if it is confirmed that its up-regulation on antigen interaction is irreversible [20]. Examination of lymphocyte history is greatly assisted by use of tetramer techniques to focus in on the peptide-sensitive cells (see below).

Three recent TG studies are of particular relevance to autoimmune disease. Wang et al. [10] created mice with TG TCRs specific for peptides from myelin proteolipid protein, peptides which are naturally present in these mice. With one TG TCR, CD4 T cells were deleted and Treg were induced in the thymus. For another, CD4 cells remained in the same functional state as those in TG mice lacking expression of this peptide. This last peptide showed low affinity for MHC and was the only one of three studied to show encephalitogenic potential. A similar state of ignorance in people could be involved in protection against multiple sclerosis (MS).

Legoux et al. [21] and Malhotra et al. [22] carried out landmark studies in which peptides from a bacteriophage or green fluorescent protein were placed transgenically in various tissues. Specific T cells from pooled lymph nodes and spleen were enriched using tetramer techniques and then analysed for possible antigenic interaction using the marker CD44. In the case of pancreas, where the epitope was introduced under the influence of the Insulin 1 gene, ignorance was found. In other tissues such as lung and intestine tolerance was maintained by deletion and/or Treg activity. These last mechanisms were associated with thymic expression of the epitopes.

3. Non-TG approach in animal models

Use of tetramer technique to enrich specific T cells has opened the possibility of studying tolerance to real self-peptides. In addition to their TG studies, Malhotra et al. [22] looked at the situation for natural self-antigens by immunisation with an appropriate peptide and concluded that the same range of tolerance mechanisms as they observed above applied to endogenous antigens. One such peptide from junctophilin 2, a protein expressed in skeletal and heart muscle, appeared to demonstrate ignorance by virtue of a strong T cell response to injected peptide and a high frequency of CD44hi cells comparable to that seen with a foreign antigen. By contrast the myelin and pancreatic peptides investigated appeared to be controlled largely by Treg activity. The pancreatic peptide studied is associated with exocrine rather than islet cells.

Earlier, Pauken et al. [19] studied CD4 T cells specific for an insulin B chain peptide in both diabetic-susceptible NOD mice and in an appropriate resistant strain. Specific CD4 cells from NOD spleen and lymph nodes showed clear evidence of antigen engagement – high CD44 expression, gamma-interferon production and markers of anergy – whereas specific cells from the control strain did not become effectors or anergic but stubbornly retained a naïve phenotype. Specific cells infiltrated the pancreas only in the NOD mice.

4. Ignorance in human T cells

The phenomenon of ignorance has been experimentally demonstrated in man in relation to a viral antigen [18]. Langerhans cells that are infected with human papilloma virus-like particles express viral peptides without becoming activated or expressing co-stimulatory molecules. Such cells will present antigen to both CD4 and CD8 T cells but only the former respond. The authors concluded that the CD8 cells remained ignorant rather than having been made anergic on the basis that the treated cells responded strongly in production of gamma-IFN on addition of activated Langerhans cells expressing co-stimulatory factors.

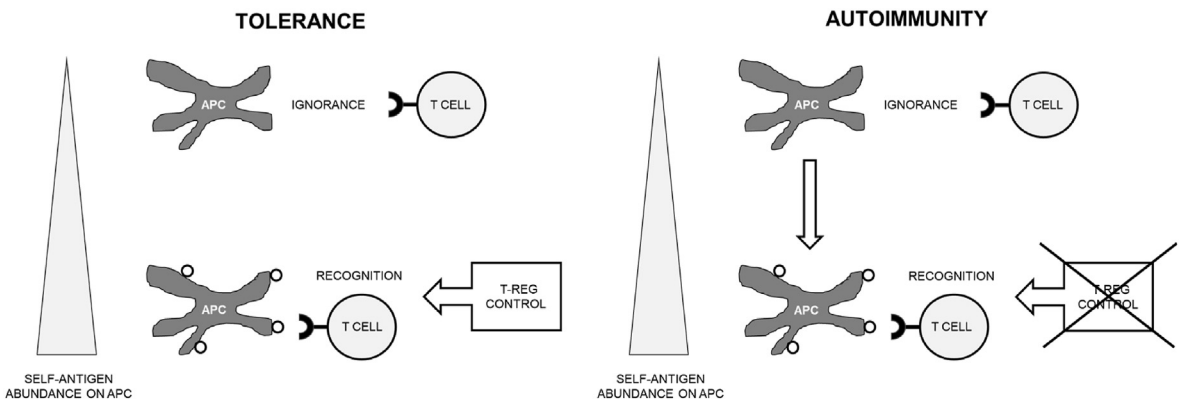

Fig. 1. Peripheral tolerance and the development of autoimmune disease
There appear to be two mechanisms of self-tolerance where T cell deletion has not occurred. In the first, top left, there is no significant expression of peptide-MHC on antigen-presenting cells (APC) so that T cells remain ignorant. In the second, bottom left, peptide-MHC is expressed on APC (shown by small open circles) but T cell activation is controlled by Treg. We are proposing that a common pathway in autoimmune disease is the breakdown of T cell ignorance consequent on a change in availability of antigen. This antigen change may be due to infectious damage of a tissue/organ or other cause as discussed. Thus, the ignorant state (top right) is transformed into that of antigen recognition (bottom right). Autoimmune disease will ensue unless Treg can deal with this new situation.

There have been several studies of T cell in vitro reactivity to type 1 diabetes (T1D) [23,24] and MS-related [25] antigens/peptides. In T1D, patient cells are found to produce gamma-IFN in response to challenge whereas cells from healthy donors are either inactive or may produce predominantly IL-10. A similar situation may exist in MS [26]. It cannot, however, be concluded from such studies that responding control cells were not naïve as the culture conditions may allow for a rapid primary reaction. Also, the controls chosen are generally matched to the patients for HLA Class II and may represent people in whom a normal state of ignorance is not secure.

In the study by Culina et al. [27] multimer enriched circulating CD8 T cells specific for a zinc transporter peptide and other islet epitopes were examined in T1D patients and controls. Phenotype was established according to expression of CD45RA, CCR7 and CDs 27, 28 and 95 and the authors concluded that the cells, at similar levels in both groups, were predominantly in the naïve state. Nevertheless, they found that the T1D cells were more likely to produce gamma-IFN on antigen challenge than the controls. There was accumulation of specific CD8 cells in the pancreas of patients.

A different picture emerges from a similar study in vitiligo [28]. Here CD8 cells specific for the vitiligo-associated antigen Melan-A were much more abundant in patients than controls. Based on a similar analysis as above, most of the specific cells in patients had memory or effector phenotype whereas in controls two-thirds were naïve. Many of the latter were, at the same time, anergic with co-expression of CD45RA, CCR7 and CTLA-4.

Systemic lupus erythematosus (SLE) may be an autoimmune disease that results from the breakdown of ignorance consequent on the impairment of the normal process of waste disposal [29]. People with the rare condition of C1q deficiency invariably develop SLE and this is almost certainly because of reduced ability to clear nucleosomal material from dying cells thus making available the antigens required for the many IgG antibodies associated with the disease. It seems then that negative selection in the thymus plays no role in protection against the disease despite the plentiful supply of nucleosomal antigens from dying cells in the thymus. There could also be a role for anergy or regulation but it is difficult to square that with the multitude of anti-nucleosomal antibodies characteristic of the disease.

5. Conclusion

The transgenic and other mouse experiments described have shown the reality and common occurrence of ignorance as a means of maintaining immune tolerance. Although data in people is much more limited at present it looks as if it will parallel that in the mouse. How important then is ignorance and its breakdown in the pathogenesis of mainstream autoimmune diseases?

It was argued above that the original sin in these diseases lies at the door of the autoantigens involved. As a result of a change in antigen on the lines described the tolerance that had been in place is now subverted and the disease may then kick off. In other words, it is not the normal tolerance arrangements that are faulty but rather their inability to adapt to a new situation. To understand what is going on it is necessary to know what the normal tolerance mechanisms are with regard to the key initiating autoantigens of the disease.

In the experiments discussed above deletion, regulation and ignorance were all commonly observed as ways of maintaining tolerance in health. Note that we are talking here about primary Treg control where ignorance does not pertain and not the possible secondary Treg activity following ignorance breakdown. By contrast, anergy was rarely encountered in this context and may be more relevant as a state that develops in disease [19]. However, due to the interchange between regulatory and anergic cells [17] one cannot rule out a role for anergy.

It looks therefore as though the failure of tolerance comes about through breakdown of ignorance or regulation. Failure of deletion could only be a factor where the change in antigen involves new antigenic determinants. Our suggestion that breakdown of ignorance is a common thread in the development of the mainstream diseases is illustrated in Fig. 1.

While there is evidence for a failure of ignorance in the case of T1D and SLE, two very different autoimmune diseases, it cannot of course be known at this stage whether all mainstream diseases will follow this pattern. Only in the case of these two diseases is there solid experimental evidence that ignorance may be involved. While other diseases may potentially show this, until similar evidence of ignorance has been obtained there is little point in further analysis. Moreover, the common finding that Treg activity is abnormally low in such diseases [30] cannot be taken as indicating that key autoantigens are not protected by ignorance in the healthy state.

If ignorance does turn out to be a general rule this may be because it is the tolerance mechanism most vulnerable to subversion. A compromise would have been made between low energy expenditure and vulnerability.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

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