OPINION

Microorganisms and autoimmunity: making the barren field fertile?

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Microorganisms induce strong immune responses, most of which are specific for their encoded antigens. However, microbial infections can also trigger responses against self antigens (autoimmunity), and it has been proposed that this phenomenon could underlie several chronic human diseases, such as type 1 diabetes and multiple sclerosis. Nevertheless, despite intensive efforts, it has proven difficult to identify any single microorganism as the cause of a human autoimmune disease, indicating that the 'one organism–one disease' paradigm that is central to Koch's postulates might not invariably apply to microbially induced autoimmune disease. Here, we review the mechanisms by which microorganisms might induce autoimmunity, and we outline a hypothesis that we call the fertile-field hypothesis to explain how a single autoimmune disease could be induced and exacerbated by many different microbial infections.

The immune system constitutes an effective bulwark against microbial disease, but it is not foolproof. In particular, during and after microbial infection, antibody and T-cell responses that are specific for self antigens can be detected; these responses are known as autoimmune or autoreactive responses. Autoreactive responses are surprisingly frequent[1], but are usually low-grade and seem to be harmless[2]. So, the detection of autoimmune (autoreactive) antibodies or T cells in an individual does not invariably imply the existence of AUTOIMMUNE DISEASE. However, in some cases, the autoreactive responses are sufficiently severe to be called AUTOAGGRESSIVE, and can result in autoimmune disease. Autoimmune diseases are thought to affect approximately 3% of US citizens[3]. For reasons that remain obscure, most autoimmune diseases are substantially (three- to fivefold) more common in women. The diseases can occur in early childhood, and the incidence increases throughout the reproductive years; they can be chronic and serious, and sometimes terminal. Examples of diseases thought to be autoimmune in aetiology include (in descending order of prevalence in the United States): Graves' disease/hyperthyroidism; rheumatoid arthritis; thyroiditis/hypothyroidism; vitiligo; pernicious anaemia; multiple sclerosis (MS); type 1 diabetes mellitus (T1D)/insulin-dependent diabetes mellitus; glomerulonephritis; systemic lupus erythematosus; and several rarer disorders, including Sjogren's syndrome, scleroderma and myasthenia gravis.

The high prevalence and frequent severity of autoimmune diseases make the exploration and understanding of the precipitating factors a clinical imperative. Autoimmune disease almost certainly involves a complex interaction between host genetics and environmental influences. Of the latter, perhaps the most important proposed initiating factor is microbial infection, and viruses, in particular, have been proposed as a cause of several autoimmune diseases in humans. For example, human T-lymphotropic virus type 1 (HTLV1) has been implicated in various autoimmune arthropathies[4]; AUTOIMMUNITY might be involved in herpes simplex virus type 1 (HSV-1) keratitis[5], although this is controversial[6]; hepatitis C virus has been proposed as a cause of myasthenia gravis[7] and various rheumatoid diseases[8,9]; and Epstein–Barr virus has been associated with systemic lupus erythematosus[10].

Of all the proposed human autoimmune diseases, perhaps the most studied in terms of infectious aetiology are T1D and MS. Several viruses (especially coxsackieviruses and rubella virus) have been proposed as causative agents of T1D[11,12], and many viruses have been proposed as the 'cause' of MS (reviewed in REF. 13). Furthermore, clinical MS exacerbations are more frequent and more severe after infection with several different microorganisms[14], indicating that nonspecific immune stimulation might be important. However, despite several decades of intensive research, no definitive evidence has emerged to identify any single infectious agent as the cause of human T1D or MS.

Viruses are not alone in being implicated in the induction of autoimmunity. Bacterial infections have also been associated with autoimmunity and autoimmune diseases. For example, group A *Streptococcus* (GAS) is associated with rheumatic fever[15] and, perhaps, with Sydenham's chorea[16]; the intestinal microflora might be associated with ankylosing spondylitis[17,18]; and Lyme disease is often accompanied by arthritis that is thought to be immunopathological in nature (reviewed in REF. 19). Furthermore, the later consequences of Chagas disease, which is caused by the protozoan *Trypanosoma cruzi*, might also have an autoimmune component[20,21]. Therefore, although this article focuses on virus-induced autoimmunity, many of the lessons and concepts can be applied to microbial infections in general. The identification of a specific microorganism as a trigger of autoimmune disease would present obvious opportunities for disease prevention and treatment — by vaccination and antimicrobial therapy, respectively.

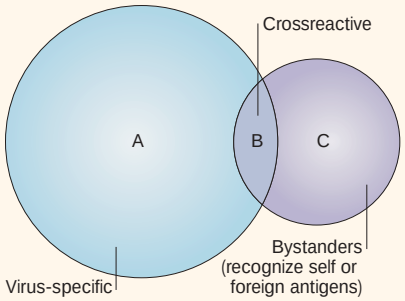

Figure 1 | **Different T-cell populations induced by virus infection.** Population A are T cells with exclusively viral specificity; these cells cannot crossreact with other antigens. Population B contains crossreactive anti-viral T cells; these cells can recognize viral antigens, and also self (or other foreign) antigens. Such T cells could be involved in molecular mimicry. Population C contains T cells with no crossreactivity to the original virus; these cells can be activated only by bystander mechanisms, which, as proposed in the text, might or might not be T-cell-receptor dependent.

Viruses and autoreactive T cells

Conceptually, there are at least two possible mechanisms underlying virus-induced autoimmunity: one invoking crossreactivity between viral and host antigens, and the other requiring no such antigenic similarity. Although the two mechanisms are usually said to be entirely distinct, we propose here that they might act in concert to induce autoimmune disease. MOLECULAR MIMICRY implies that similar epitopes are shared between the virus and the host[22]; the virus antigen should be sufficiently different from the host antigens to initiate an immune response, but sufficiently similar that the induced response is crossreactive. The resulting 'anti-host' response could then be maintained even after clearance of the virus. Alternatively, a virus infection might induce and/or activate $CD8^+$ T cells that are not specific for that virus — that is, which cannot recognize epitopes that are contained in the viral proteins. The absence of crossreactivity between the infectious agent and the responding lymphocytes has led to these cells being called 'bystanders', and this mechanism of virus-induced autoimmunity is known as BYSTANDER ACTIVATION.

These concepts are represented by the Venn diagram in FIG. 1. In theory, a virus infection can induce T cells of three specificities: cells that react only with viral epitopes (population A); cells that react only with epitopes absent from the virus (population C); and crossreactive cells, which recognize epitopes that are common to both viral and host proteins (population B). This diagram shows clearly that the potentially autoreactive cells (populations B and C) can be divided into two conceptual classes, which correlate with the two general mechanisms by which microorganisms might induce autoimmunity (described above). Population B can crossreact with the virus, and so can be induced by molecular mimicry, whereas population C cannot recognize viral antigens and therefore must have been induced by bystander mechanisms. To what extent are each of these two mechanisms supported by experimental evidence?

T-cell activation by molecular mimicry?

Almost a decade ago, two of us (R. S. F. and J. L. W.) attempted to demonstrate virus-induced molecular mimicry by expressing a self protein from the rat central nervous system (CNS) in a recombinant vaccinia virus; we showed unequivocally that infection of mice with this recombinant virus rendered these animals much more susceptible to the autoimmune disease experimental autoimmune encephalomyelitis[23]. We have recently shown that immunization with DNA encoding the same protein has a similar effect[24]. The development of transgenic technology allowed the converse course to be taken; instead of expressing a host gene in a virus, it became possible to express a viral gene product in the host, in a relatively tissue-specific manner, and thereafter to determine if mice bearing this new 'viral-self' protein were susceptible to autoimmune disease triggered by infection with the appropriate virus. One of the best-studied transgenic models uses the nucleoprotein (NP) or glycoprotein (GP) of the lymphocytic choriomeningitis virus (LCMV) Armstrong strain expressed under the control of the rat insulin promoter (RIP), which directs transcription in the β-cells of the ISLETS OF LANGERHANS[25,26]. In these mice, the LCMV protein is, in essence, a self protein, and seems to be non-toxic to the islets as, in the absence of LCMV infection, T1D is rare (<5%). However, infection of RIP transgenic mice with the LCMV Armstrong strain frequently (>95%) triggers diabetes, which can be 'fast-onset' (~14 days post infection) or 'slow-onset' (2–6 months post infection)[27]. Disease seems to require the destruction of islet cells by NP- or GP-specific $CD8^+$ T cells, and the number of autoreactive cells is crucial; a recombinant vaccinia virus expressing an identical LCMV NP molecule cannot trigger diabetes despite inducing NP-specific $CD8^+$ T cells because the number of cells that are induced falls below the threshold for disease induction[28]. T-cell effector functions are also important; in the absence of PERFORIN[29] or interferon-γ (IFN-γ)[30], T1D does not occur, even after infection with the LCMV Armstrong strain. Finally, the status of the target cell is crucial; additional accessory molecules such as B7.1 greatly increase the susceptibility to T1D, in some cases resulting in spontaneous disease, that is, without infection[28].

Strictly speaking, these studies do not represent molecular mimicry, the concept of which is that the host and virus share similar, rather than identical, antigens. For true molecular mimicry therefore, the T-cell receptor (TCR) on the responsible T cells must be able to recognize two different peptide epitope

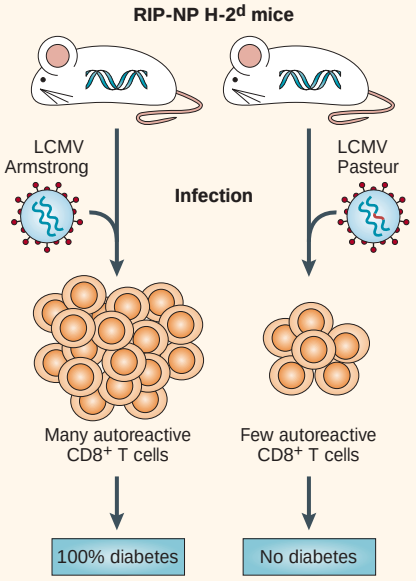

Figure 2 | **Molecular mimicry and diabetes.** Recent data have shown that molecular mimicry alone does not precipitate diabetes. RIP-NP H-2^d mice express the nucleoprotein (NP) of the lymphocytic choriomeningitis virus (LCMV) Armstrong strain under control of the rat insulin promoter (RIP) — the viral protein is therefore expressed in the β-cells of the islets of Langerhans in the kidney. Infection of RIP-NP H-2^d mice with the LCMV Armstrong strain induces type 1 diabetes within 2 weeks[27,33]. By contrast, infection with the LCMV Pasteur strain induces no autoimmune disease. The sequence of the immunodominant NP_{118} CD8 cytotoxic T lymphocyte (CTL) epitope differs between the two viral strains[32] and, interestingly, the Pasteur epitope is still presented by major histocompatibility complex (MHC) class I, but elicits a significantly lower number of $CD8^+$ CTLs systemically, owing to its lower avidity. The number of CTLs induced by LCMV Pasteur was high enough to clear the viral infection, but too low to trigger autoimmunity to the NP antigen. So, a true viral mimic was incapable of *de novo* induction of autoimmune disease under conditions where the native epitope could.

sequences: one from the microorganism and the other from the host. The ability of an individual TCR to recognize more than one epitope sequence is known as 'TCR degeneracy', and it is abundantly clear that TCRs are, in general, somewhat more degenerate (that is, less sequence-specific) than was originally surmised[31]. Therefore, our present understanding of T-cell responsiveness renders molecular mimicry plausible, at least in theory. However, some recent data, summarized in FIG. 2, indicate that true molecular mimics, in some circumstances, are unable to induce disease. In these experiments, RIP-NP mice, which express the NP from the LCMV Armstrong strain, were infected with a closely related LCMV strain (LCMV Pasteur), which differs from the LCMV Armstrong strain by a few amino acids in the NP epitope; T cells induced by the LCMV Pasteur strain have a reduced AVIDITY for the Armstrong NP epitope[32], and the LCMV Pasteur strain failed to induce T1D[33]. In summary, the evidence that molecular mimicry can induce disease remains controversial even in well-defined and manipulable animal models (reviewed in REF. 19); it is therefore not surprising that its relevance to human disease still remains uncertain.

Perhaps the best-characterized correlation in humans is between GAS infection and heart disease; bacterial *N*-acetyl glucosamine induces antibodies that seem to crossreact with the vascular endothelium, leading to inflammation, recruitment of T cells and damage to the myocardium and heart valves[15]. The evidence in animal models is becoming more compelling, and recent data indicate that cross-reactive T cells that are specific for the bacterial M PROTEIN might attack host tissues[34], but the relationship in humans remains correlative; definitive data proving cause and effect are elusive. There are several other interesting correlations in humans. A striking recent example is the association between rotaviral infections and the presence of autoantibodies to insulin and glutamic acid decarboxylase (GAD) in a group of young children in Australia[35].

Several lines of experimental evidence indicate that molecular mimicry alone might be incapable of causing clinical disease, and can do so only in conjunction with other factors. These could be provided, for example, by virally induced inflammation or a nonspecific inflammatory process. When genetically susceptible mice (SJL/J strain) are infected with a recombinant vaccinia virus encoding a self protein (that is a molecular mimic), these mice neither exhibit clinical signs of autoimmune disease nor have pathological lesions within the CNS. However, these mice have been primed for disease; if, weeks later, they receive a nonspecific inflammatory stimulus – for example, complete Freund's adjuvant (CFA) – 80–90% of the mice develop disease[36]. Interestingly, only certain viral infections can provide a sufficient inflammatory stimulus to cause disease (R. S. F. and I. Tsunoda, unpublished observations); when primed mice are infected with LCMV, no disease is observed, but murine cytomegalovirus infection leads to CNS inflammatory lesions in more than 60% of the mice. In another experimental system, a recombinant Theiler's virus expressing an epitope mimic of a CNS protein can lead to enhanced experimental autoimmune encephalomyelitis[37]. It is likely that viral persistence is important in this model, sustaining the CNS autoreactivity, perhaps through activation of antigen-presenting cells (APCs). So, molecular mimicry results in disease only when there is appropriate secondary stimulation. These considerations have led us to propose that a 'fertile field' might be required to allow autoreactive T cells, induced by molecular mimicry, to become autoaggressive.

Glossary

ANTIGEN-PRESENTING CELL
A cell that is capable of providing co-stimulation through CD80/86, CD40 and other mechanisms, in addition to presenting antigen in the context of MHC molecules. Examples include dendritic cells, B cells and macrophages.

AUTOAGGRESSIVE
A term that is used to describe autoreactive T cells that are harmful to the host.

AUTOIMMUNE DISEASE
A clinically apparent disease that is attributable to the activities of autoaggressive lymphocytes.

AUTOIMMUNITY
An antigen-specific response against host tissues that is equivalent to autoreactivity. Note that many autoimmune and autoreactive lymphocytes seem to be harmless, or even protective to the host.

AVIDITY
The strength with which a T cell binds to a target cell by multiple receptor–ligand interactions.

BYSTANDER ACTIVATION
The activation of autoreactive lymphocytes that do not recognize microbial antigens. This can be mediated through cytokines and/or APCs.

COGNATE ANTIGEN
An antigen for which an antibody, or a T-cell receptor, is specific.

ISLETS OF LANGERHANS
Clusters of cells that are found in the pancreas. There are five types of islet cells, including β cells, which make insulin; α cells, which make glucagon; and δ cells, which make somatostatin.

MOLECULAR MIMICRY
This can occur when lymphocytes that are induced by a foreign (usually microbial) antigen can recognize (crossreact with) host proteins. Usually, the avidity of the lymphocytes for the self antigen is lower than their avidity for the microbial antigen.

M PROTEIN
A streptococcal virulence factor that protects the bacteria from phagocytosis.

PERFORIN
A pore-forming protein that is present in the granules of cytotoxic lymphocytes.

RANTES
A CC-chemokine that binds to, and activates, signal transduction by several chemokine receptors.

T-cell activation by bystander effects?

These effects are often characterized as being non-antigen specific and cytokine driven. However, we consider it likely that a substantial component of bystander activation is antigen specific, and therefore we suggest that bystander effects are better divided into two categories: TCR independent and TCR dependent.

TCR-independent bystander activation. This is the classical meaning of the term bystander activation; it is non-antigen specific, and so does not depend on T-cell stimulation by the TCR; instead, it is driven by pro-inflammatory cytokines and chemokines that are commonly produced during viral infections, for example, interferons, interleukin (IL)-12, RANTES and inflammatory protein (IP)-10 (REF. 38). For many years, it was thought that more than 95% of the $CD8^+$ T cells that are induced by viral infection were induced in this manner, and some data were available to support this view[39,40]. However, several seminal papers have shown beyond doubt that, during virus infection, most of the responding $CD8^+$ T cells are not bystanders, and are specific for viral epitopes; this seems to apply to both mice and humans[41–43]. Although TCR-independent bystander activation cannot be completely discounted[44–47], it seems that cytokines alone are unlikely to drive extensive expansion of, or functional changes in, T cells in the absence of their COGNATE ANTIGEN[41,48–55]. So, we must seek an additional, or different, explanation for bystander activation.

TCR-dependent bystander activation. In contrast to the generally accepted hypothesis, we suggest that, in most cases, bystander activation might require concurrent exposure to

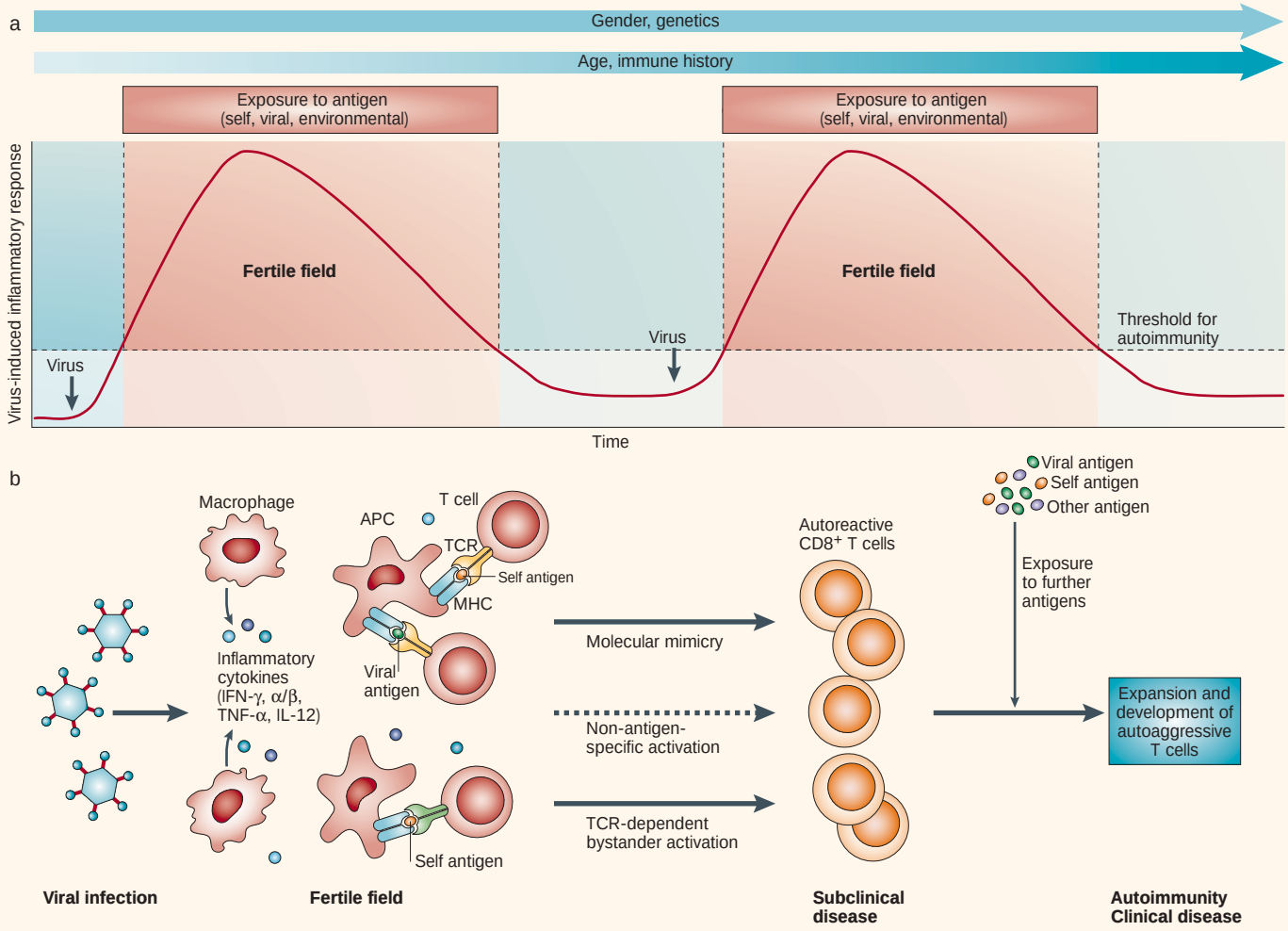

Figure 3 | **The fertile-field hypothesis. a** | A diagrammatic representation of the fertile-field hypothesis. The top arrow represents gender and genetics, which are relatively constant throughout the lifetime of the host. By contrast, the factors represented by the lower arrow (age and immune history) are constantly changing. The fertile field is shown as a temporary period that follows virus infection, and which can vary depending on the type, anatomical location and duration of the virus-induced inflammatory response. **b** | The fertile-field hypothesis and virus-induced autoimmune disease. The fertile-field hypothesis explains how viral infections can induce and/or expand autoreactive T cells, and can cause them to become autoaggressive, leading to clinical autoimmune disease. Infection with the right virus at the right time creates a transient, localized fertile field. The autoreactive cells generated can either crossreact with viral antigens (molecular mimicry) or can react only with autoantigens (bystander activation). In the former case, virus-specific cells have the potential to become activated directly and accelerate the development of clinical disease. In the latter case, an intermediate mechanistic link that causes activation of autoaggressive bystanders in the presence of a viral infection with entirely different antigenic specificity is postulated. As described in the text, such bystander activation probably occurs through professional or non-professional ANTIGEN-PRESENTING CELLS (APC) that process and present self antigen (determinant spreading). Pro-inflammatory cytokines and chemokines also might contribute to non-antigen-specific (TCR-independent) bystander activation, although we believe the contribution of this form of bystander activation is minor. IFN, interferon; IL-12, interleukin-12; MHC, major histocompatibility complex; TCR, T-cell receptor; TNF-α, tumour necrosis factor-α.

the cognate antigen. For example, the data presented above indicate that antigen-specific cells that are induced by molecular mimicry (that is, population B in FIG. 1) can be activated by a nonspecific immune stimulus (CFA, poly-IC or some virus infections) to induce autoimmune disease. The widely accepted explanation for this result is that the nonspecific stimulus induces cytokines, and that these alone are responsible for activating the autoreactive cells. We propose that, in addition to the cytokines, a second signal is required for full activation of the autoreactive cells, and that this signal is contact between the TCR and the cognate self antigen. Therefore, in the above example, we argue that molecular mimicry and bystander activation can interact; autoreactive cells are induced by molecular mimicry, but the development of autoaggression (and resulting disease) requires their subsequent activation by bystander means. The importance of inflammatory cytokines for the pathogenesis of autoimmunity is well documented, and administration of cytokines can induce relapse of disease[56] but, in striking similarity to the observations concerning molecular mimicry, there are very few examples where inflammatory cytokines alone can break tolerance to autoantigens in healthy animals, thereby causing clinical disease. In most cases, local overexpression of cytokines or chemokines leads to inflammation but not clinical disease. This is true for IL-2, IL-12 and IP-10 in both diabetes and MS models (REFS 57,58; U. Christen, M. Oldstone and M. G. V. H., unpublished observations). One exception is IFN-γ, which, when overexpressed by pancreatic islet β cells, disrupts tolerance to a defined autoantigen[59]. We propose that the harmful effects of IFN-γ might be related to the effects of this cytokine on antigen presen-

tation: IFN-γ can upregulate the presentation of self antigens, so completing the strong activation signal that is required to drive autoreactive T cells into autoaggressive mode[60]. So, we can again conclude that several factors contribute to bystander-mediated disease, a realization that strongly supports the fertile-field concept.

In the above example, we describe how bystander effects could activate pre-existing autoreactive cells, induced by molecular mimicry. However, the induction of autoreactive cells does not necessarily depend on molecular mimicry; it is possible that the entire process (induction and subsequent activation) could be driven by bystander effects, which could induce cells that do not crossreact with viral antigens. This could happen if host proteins, which are released during the infection, were captured by specialized APCs that could process and present host epitopes; if these epitopes were immunogenic, the resulting activated T cells would recognize self proteins, but would not crossreact with viral epitopes (that is, population C in FIG. 1). Note that, regardless of whether the autoreactive cells were induced by molecular mimicry (population B), or by the presentation of self epitopes (population C), one main attraction of these ideas is that subsequent infection by a broad range of microorganisms might activate the autoreactive cells, therefore explaining why several different microorganisms can exacerbate any given autoimmune disorder.

The fertile-field hypothesis

As described above, there are well-defined notions of how T cells might cause autoimmune disease, and several animal models are available in which these theories can be tested. Why, then, has it proven impossible to identify any virus as the cause of any human autoimmune disease? In addition to the magnitude and quality of the autoreactive response, it seems to us that time is of the essence — it is important to understand that the priming (induction) event can be separated greatly in time from the subsequent activation events. For example, epidemiological data, including migrant studies[61], indicate that early exposure to infectious agents can confer susceptibility to MS in later years; this is consistent with the notion that the induction phase (be it by molecular mimicry or by bystander induction) occurs at an early age and the transition to autoaggression and disease — driven, possibly, by a new infection with any one of several different microorganisms, with no requirement that they encode crossreactive antigens — can occur years later. It is, of course, possible that more than one subsequent infection is needed to amplify the number and/or activation status of the autoreactive cells, eventually leading to frank disease. So, we feel that it is important to include time in the equation when evaluating how various immunological stimuli might contribute to autoimmune disease. The importance of temporal relationships is further underscored by considering the possible link between virus infection and allergic disease. Clinical observations[62] and experimental studies[63] indicate that, in a previously non-allergic host, sensitization to a respiratory allergen is more likely to occur if the host has an ongoing virus infection at the time of allergen exposure.

Taken together, the above ideas indicate that, as suggested previously[64], microbial infection (or even the administration of powerful adjuvants[36]) can induce a temporary immunological state for which we propose the term 'fertile field'. If this short-lived field is 'sown' with other antigens (environmental, viral or self; the latter will vary depending on the host genotype), it can yield a pathogenic 'harvest' of autoreactive or autoaggressive T cells. This viewpoint clarifies why microorganisms should be seen as factors contributing to, rather than being the sole causative agents of, autoimmune (and allergic) diseases. Organ-specific autoimmune diseases such as T1D and MS are most commonly associated with viruses that replicate extensively in the affected tissue, consistent with the idea that infection induces a transient, and localized, fertile field — 'fertilizing' the growth of organ-specific lymphocytes.

The fertile-field hypothesis is presented in graphical form in FIG. 3. As indicated, a conceptual threshold for autoimmune disease can be exceeded if several factors coincide. Host gender and genetics are (for the most part) consistent throughout an individual's life. However, there are (at least) three variables that must be considered. First, the fertile field that is induced by virus infection is transient, and will change quantitatively and qualitatively over the course of infection. Second, the age and immunological history of the host are constantly changing, and these can modulate how the host immune system responds to antigens encountered in the fertile field. Third, the antigens present in the field will vary depending on: the anatomical location of the fertile field (where the field is situated); the infectious agent involved or other environmental factors, for example, allergens, to which the host will be exposed only sporadically, often depending on the time of year (what foreign seeds are planted); and the precise cytokine milieu (the amount of fertilizer used), which can dictate the degree to which host antigens are processed and presented.

"…microbial infection can induce a temporary immunological state for which we propose the term fertile field."

Box 1 | Difficulties in associating viruses and autoimmune diseases

Hit-and-run events
As the viral nucleic acid has been cleared by the time of diagnosis of autoimmune disease, a causative association is hard to prove.

Persistent infections
Latency can be an important problem in finding the agent unless biopsy of the target organ is a possibility.

Viral strains
Different strains of the same virus vary in the immune responses they generate, which might alter their effects on ongoing autoimmunity.

Timing of infection
The pre-clinical autoimmune process undergoes distinct phases that can vary in their susceptibility to viral interference.

Abrogation of autoimmunity by infections
Viral infections can ameliorate autoimmune disease.

Regulatory T cells (Tregs)
Viral infections might, in certain instances, stimulate or activate lymphocytes that recognize autoantigens, but have effector functions that dampen the autoaggressive response, rather than exacerbating it.

Table 1 | Age-dependent effects of CVB3 infection on TID in NOD mice

Age when infected with CVB3 (weeks)	Effect on T1D	References
4	Amelioration	67
10–12	Acceleration	47
16	Amelioration	67

CVB3, coxsackievirus B3; NOD, non-obese diabetic; T1D, type 1 diabetes.

The fertile-field hypothesis is attractive for several reasons. First, we might be able to identify certain common inflammatory responses to otherwise distinct pathogens that result in a given autoreactive or autoaggressive response. Second, the fertile-field concept might explain why factors such as inflammatory cytokines, or mechanisms such as molecular mimicry, rarely precipitate disease on their own in otherwise healthy hosts. Third, the requirement that virus infection and antigen exposure be concurrent could explain why either event alone often fails to induce allergic responses. These ideas indicate that searching for specific infectious agents in association with a given autoimmune or allergic disease might be futile. Rather, we should develop a better understanding of the inflammatory factors and principal pathways that are associated with enhancement of autoimmune disease in experimental models. This knowledge will enable us to detect similar signs in individuals at risk and, ideally, implement appropriate preventive measures.

Difficulties in establishing a viral cause

Several factors have contributed to our failure to identify any virus as the 'cause' of autoimmune diseases such as T1D and MS (BOX 1). First, an acute viral infection can induce autoreactive cells, but most infections are cleared within weeks — long before autoreactive cells become autoaggressive. By the time clinical autoimmune disease is diagnosed (months or years later), the viral sequences have been eliminated. Second, the detection of antiviral antibodies or T cells in patients with autoimmune disease could provide a correlation; however, some virus infections are extremely common and traces of these infections can be found in almost every individual — most of whom will not develop autoimmune disease. This is the situation for Epstein–Barr virus, which has been proposed as a trigger of MS, but which can be identified in more than 95% of adults. It will therefore be extremely difficult to identify a single virus as a cause by measuring antiviral antibodies or T cells, and it might be essential to identify the precise viral strain, the timing of infection and the magnitude of infection in a prospective clinical study. Third, it is possible that, in individuals carrying autoreactive cells, clinical disease might first appear soon after a fertile field was present. Unfortunately, as explained above, an appropriate fertile field could be produced by any one of several different infections, rendering it difficult to draw a clear relationship between one virus and clinical disease, even when the two were quite closely related in time. Fourth, as noted above when comparing the closely related LCMV strains Armstrong and Pasteur, the ability of a virus to induce autoimmune disease can be markedly altered by changes in a few amino acids. So, the identification of virus–disease relationships might require extensive sequencing of viral genomes from numerous diseased and healthy individuals. Fifth, in mice known to have pre-existing autoreactive cells, the immunological effects of a fertile field might not always promote autoimmunity; in some cases, the converse might occur. For example, tumour necrosis factor (TNF) accelerates T1D when expressed early in the disease process, but dampens autoimmunity when expressed relatively late[65,66]. Further complicating matters, the effect of virus infection can oscillate depending on the age of the animal, as shown by the effects of coxsackievirus B3 (CVB3) infections on the development of T1D in non-obese diabetic (NOD) mice at different ages[67].

The data are summarized in TABLE 1. Infection with CVB3 commonly leads to amelioration of T1D in young and old NOD mice, but infection of 10–12-week-old female NOD mice accelerates disease. Although it is known that this acceleration probably occurs through bystander effects of the virally mediated inflammation on the autoaggressive T-cell repertoire[47], it is unclear why amelioration of T1D is observed at most ages. Finally, virus-induced molecular mimicry can induce cells that protect against the subsequent development of autoimmune disease. Some time ago, it was shown that a recombinant virus expressing a known encephalitogenic sequence from myelin basic protein not only failed to enhance disease susceptibility, it instead protected against experimental autoimmune encephalomyelitis, showing that it is possible to vaccinate against autoimmune diseases[68]. This concept was confirmed in the animal model of T1D; a DNA vaccine encoding insulin conferred protection against virus-induced disease by inducing autoreactive regulatory cells[69,70]. These findings are consistent with the observation that autoimmune diseases are quite rare in countries with a high incidence of infectious disease; for example, T1D and MS are infrequent in equatorial countries. Therefore, the consequence of exposure to self antigens is variable, is at present unpredictable and probably depends on the antigen chosen, and the timing and nature of antigen delivery.

Future directions

This article presents a flavour of the enormous complexity of the association between viral infections and autoimmunity. Where does this leave us? The various interactions that can occur between viruses, APCs, the fertile field and molecular mimicry are summarized in FIG. 3b and, we believe, should shape our thinking about virus-induced autoimmune diseases. We should try to discover commonalties between viruses with respect to the type and magnitude of inflammation they induce. This could be achieved, for example, by using proteomic approaches on human blood or assessing specific metabolic changes during acute infections. In certain experimental models, the proteome profile that is present in blood can predict, on an individual basis, whether autoimmune disease will develop or not after intervention[71]. We propose to classify the fertile field in a similar fashion. Ultimately, the type of inflammation, coupled with the precise timing of infection, might best correlate with a positive or negative effect on autoimmune disease.

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