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The potential role for infections in the pathogenesis of autoimmune Addison's disease

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Summary

Autoimmune Addison's disease (AAD), or primary adrenocortical insufficiency, is a classical organ-specific autoimmune disease with 160 years of history. AAD is remarkably homogeneous with one major dominant self-antigen, the cytochrome P450 21-hydroxylase enzyme, which is targeted by both autoantibodies and autoreactive T cells. Like most autoimmune diseases, AAD is thought to be caused by an unfortunate combination of genetic and environmental factors. While the number of genetic associations with AAD is increasing, almost nothing is known about environmental factors. A major environmental factor commonly proposed for autoimmune diseases, based partly on experimental and clinical data and partly on shared pathways between anti-viral immunity and autoimmunity, is viral infections. However, there are few reports associating viral infections to AAD, and it has proved difficult to establish which immunological processes that could link any viral infection with the initiation or progression of AAD. In this review, we will summarize the current knowledge on the underlying mechanisms of AAD and take a closer look on the potential involvement of viruses.

Keywords: Addison's disease, autoimmunity, chemokines, virus infections

Introduction

Appropriate production of steroid hormones by the adrenal cortex is vital for human life. Mineralocorticoids such as aldosterone regulate the body's salt and water balance, while glucocorticoids such as cortisol are important for many aspects of metabolism and for mobilization of energy during stressful conditions. In the adrenals of patients with autoimmune Addison's disease (AAD), the steroid hormone-producing cells of the adrenal cortex are targeted by an immune-mediated attack resulting in organ failure and lack of steroid hormone production [1,2]. Patients with AAD therefore depend upon lifelong supplementation therapy which does not restore full health, but leaves patients exposed to increased morbidity, heightened mortality and reduced quality of life [3–6]. There is therefore a need to improve understanding of the underlying pathological mechanisms behind AAD in order to form the basis for rational design of molecular and cellular strategies that target the genesis of the disease. The development of AAD and most autoimmune diseases is probably a multi-factorial process involving a combination of genetic and environmental factors and the failure to control autoreactive lymphocytes at multiple stages [7]. Several genetic risk factors are known, in particular certain variants within the major histocompatibility complex (MHC) [6,8] but almost nothing is known about environmental factors.

While AAD may occur in isolation, more than half the patients suffer from additional autoimmune diseases, frequently autoimmune thyroiditis (hypo- or hyperthyroidism) and type 1 diabetes [6,9,10]. The co-existence of two of these three is commonly referred to as autoimmune polyendocrine syndrome type 2 (APS-2). AAD is also a major

disease component of autoimmune polyendocrine syndrome type 1 (APS-1), in which the presence of two of the following three manifestations is diagnostic: AAD, chronic mucocutaneous candidiasis and hypoparathyroidisim [11]. While the two syndromes share several features such as the presence of serum adrenal autoantibodies and immune infiltration of the adrenal cortex [12], the genetic background of APS-1 differs from APS-2 as the former is caused by mutations in the AIRE (autoimmune regulator) gene, and APS-2 (including isolated AAD) is a polygenic disorder. AIRE plays a critical role in the negative selection of immature T cells in the thymus, preventing the escape of autoreactive T cells and development of autoimmunity [13]. Hence, mutations in AIRE lead to multi-organ autoimmunity. The mechanisms by which AAD develops in APS-1 and APS-2 are therefore thought to differ. Thus, we will focus mainly on the latter form here.

A recent twin study from Sweden revealed that the heritability of AAD is extremely high, indicating a strong genetic influence on the occurrence of disease [14]. However, although the concordance rate of monozygotic twins is high at 0·73, the involvement of additional variables such as environmental factors is very likely. This is supported further by an apparent increase in the incidence of AAD in Europe during recent decades [15–19]. Knowledge of AAD is scarce in other parts of the world, but epidemiological data indicate that the prevalence is exceedingly far lower in the Far East and South Africa than in Europe [20–22]. However, the same major histocompatibility complex (MHC) risk alleles are shared between European, North American and South African patients [23], indicating additional environmental factors. Of course, these may include protective as well as predisposing factors, including childhood infections that may actually protect against autoimmune diseases [24]. Nevertheless, suspected environmental triggers in autoimmune diseases include infectious agents such as viruses, bacteria and other pathogens, and these will remain the focus of this current review [25]. Unfortunately, unlike certain other autoimmune diseases such as type 1 diabetes, multiple sclerosis or autoimmune liver diseases [26–28], no specific candidate infectious agents have been identified for AAD. However, many infectious pathogens are known to infect the adrenal cortex and some of them may also cause adrenal insufficiency [29]. In the following we will review some of these infectious agents, and highlight how they may take part in the pathogenesis of AAD.

Microbial causes of adrenal insufficiency

In earlier years, adrenal insufficiency was caused predominantly by infection with *Mycobacterium tuberculosis*, which spreads from the lungs to the adrenals, causing necrosis and calcification [30]. However, this cause of adrenal insufficiency is now generally confined to regions where the bacterium is still endemic, and is not associated with the autoimmune form that dominates in developed countries today. When considering that an infection could serve as a trigger for adrenal autoimmunity, a variety of other microorganisms has also been shown to infect the adrenal gland and could be of interest [29]. These include a diverse array of viruses, bacteria, fungi and parasites. However, we find viruses to be the most relevant, given the immune responses involved in AAD, the frequent links to other autoimmune endocrinopathies (such as type 1 diabetes), and the fact that many of the other microbes with a tropism for the adrenals, such as *M. tuberculosis* or *Paracoccidioides brasiliensis*, are not endemic in the western world [31,32]. The latter pathogens are also examples of agents capable of mediating direct adrenal destruction. Conversely, viruses are often capable of establishing chronic or latent infections in humans. Such chronic infections often have features typical for autoimmune diseases, such as autoantibodies and organ-specific inflammation.

Many of the infectious agents known to infect the adrenal cortex and cause adrenal insufficiency are opportunistic infections, primarily affecting immunocompromised individuals. In particular, patients with acquired immunodeficiency syndrome (AIDS) caused by HIV are susceptible to adrenalitis. In a morphological evaluation of adrenal autopsies from 128 patients dying from AIDS, more than 99% had adrenalitis, with cytomegalovirus (CMV) being the cause in half the cases [33]. Bacterial, fungal and protozoan infectious agents such as *Mycobacterium* sp., *Histoplasma* and *Cryptococcus* sp. and *Tryponosoma* and *Toxoplasma* sp. were also detected. Other studies have reported that the adrenal gland is the most frequently CMV-affected organ in AIDS patients [34]. CMV and adrenalitis have also been described in other acquired immunodeficient states, such as allogeneic haematopoetic stem-cell transplantation, kidney transplantation and liver transplantation after chronic hepatitis C virus infection [35–38]. Finally, adrenal infections by CMV and also other members of the *Herpesviridae* family, such as herpes simplex virus types 1 and 2 (HSV-1/HSV-2) and human herpesvirus 6 (HHV-6), have been reported in neonates and infants [39–42]. Although the children described with these infections were seemingly immunocompetent (e.g. no signs of concomitant HIV infection or genetic causes of severe immunodeficiency), the immune systems of neonates and infants are immature with suboptimal responses to infections and vaccines [43,44]. Viral adrenalitis in primary immunodeficiencies have also been described, including adrenal insufficiency caused by

Epstein–Barr virus (EBV) infection in an adolescent with Wiscott–Aldrich syndrome and subclinical adrenal CMV infection discovered at autopsy in children with severe combined immunodeficiency [45,46]. However, some of the viruses described above, including HSV-1 and CMV, have also been associated with adrenalitis in apparently immunocompetent adults [29,47,48]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections have also been reported in connection with adrenal insufficiency [49,50]. Interestingly, in one patient hepatitis B surface antigen was detected in autopsy material from remaining adrenal tissue, indicating that HBV can have a tropism for the adrenal cortex [49].

The susceptibility of the adrenals to viral infections in immunosuppressed or immunodeficient individuals is mirrored by reports suggesting impaired immunity and increased susceptibility to infections in patients with AAD. Recently it was found that AAD patients have impaired natural killer (NK) cell functions, potentially compromising their early recognition and elimination of virus-infected cells [51]. Furthermore, it has been demonstrated that peripheral blood cells from AAD patients respond poorly to *in-vitro* stimulation with interferons (IFNs), which substantiates the notion of impaired early anti-viral immune responses [52]. Epidemiological investigations have also suggested that AAD patients have more infections, and are prescribed with more anti-microbial agents (including anti-virals), than the general population [15,53]. However, the interpretation of these data is complicated by the fact that AAD patients are medicated with exogenous glucocorticoids that have many immunomodulatory effects [54]. Although AAD patients have little to no endogenous glucocorticoid production and replacement doses are attempted to be kept within physiological borders, it is recognized that excessive use of glucocorticoids increases the risk of infectious complications [55]. It is therefore unclear whether the increased risk of infections in AAD patients is related to glucocorticoid replacement therapy or to a partial immune defect. Importantly, however, the increased susceptibility to infections in AAD patients does not show a clear relationship with glucocorticoid dosage, and is present already in incident patients prior to any glucocorticoid treatment [53]. In a Danish nationwide study investigating more than 4·5 million people born between 1945 and 2000, an association between infection-related hospital admissions and subsequent diagnoses of 29 different autoimmune diseases was found [56]. AAD was among the diseases with the strongest association to hospitalization for serious infections prior to diagnosis. Intriguingly, for AAD in particular, an increase in the number of infections increased the risk for autoimmune disease in a dose-dependent manner with patients having five or more infections. However, a word of caution is needed when interpreting these data. Serious infections (e.g. involving sepsis) require rapid activation of adrenocortical glucocorticoid production as a fundamental part of the stress response [57]. As AAD can have a long subclinical phase with adrenal impairment, infections requiring rapid glucocorticoid production may easily precipitate clinically overt adrenocortical failure [12]. It is therefore possible that the increased number of infections in AAD patients prior to diagnosis is merely reflecting the increased severity of infections in individuals with impaired adrenal function, and that these infections are unmasking the adrenocortical insufficiency rather than causing it. Indirect support for a role for infections early in life does, however, exist. A recent study from the United Kingdom and Poland demonstrated that month of birth exerts an effect on the risk of developing AAD, with peaks in December and January and troughs in May and July, suggesting increased exposure to seasonal viral infections in the perinatal period in individuals who develop AAD later in life [58].

Some studies have also suggested that the adrenal cortex is a natural reservoir for several viruses that are able to establish latent infections in humans, and that excessive glucocorticoid production could reactivate these viruses [59,60]. Viral agents detected in both normal adrenals and adrenocortical tumours (both benign and malignant) included most members of the *Herpesviridae* family and the polyomaviruses SV40 and BK virus. Primary infections with herpesviruses and polyomaviruses usually occur early in childhood, and thereafter these viruses establish latent or persistent infections that may be reactivated by immunosuppression. Interestingly, experimental infections with HSV-1 in mice have revealed that the adrenal cortex is among the first organs to be infected before spreading of the virus to the central nervous system [61]. In mice surviving primary HSV-1 infection, subcapsular lesions developed in the adrenal cortex and persisted for up to 1 year after inoculation [62]. These findings suggest that infections early in life, such as primary HSV-1 infections, may leave lesions and scarring behind in the adrenal tissue, perhaps rendering individuals more susceptible to develop AAD later in life.

Gene–environment interactions

Several genetic factors that increase the risk of developing AAD have been identified, most of which play important roles in the interactions between antigen-presenting cells (including B cells) and T cells [63]. This is very typical for organ-specific autoimmune diseases and many genetic risk factors for AAD are, in fact, shared with related

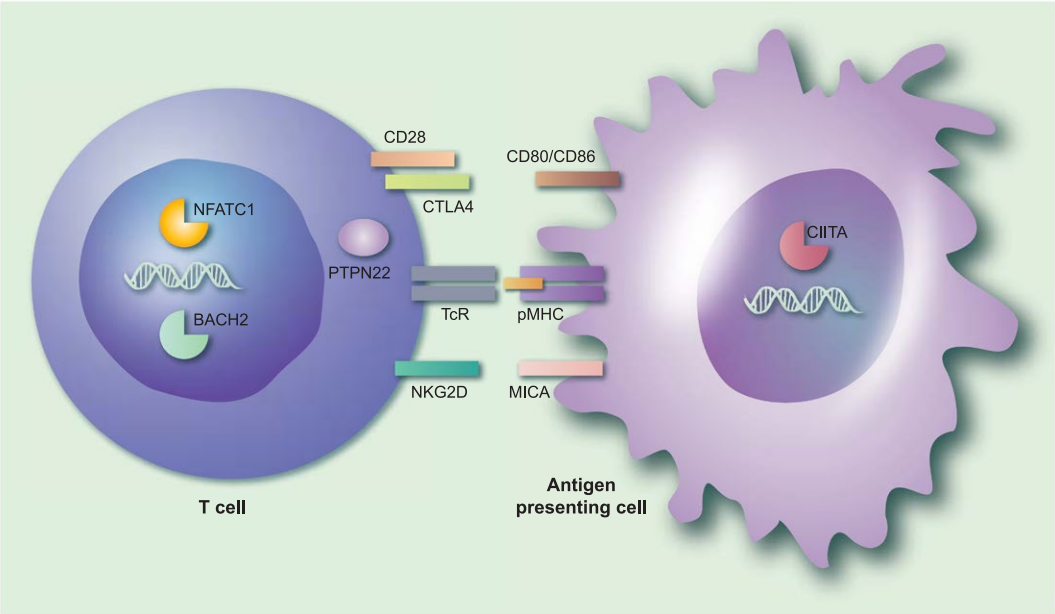

Fig. 1. The immunological context of genetic susceptibility factors for autoimmune Addison's disease that may interact with infectious agents. The figure gives an overview of the immunological processes implicated by the genetic susceptibility factors associated with autoimmune Addison's disease (AAD). Note that both the products encoded by the risk genes themselves (denoted in bold type below) and their interaction partners are shown in the figure. Professional antigen-presenting cells, such as dendritic cells or activated B cells, present antigenic peptides (from self or from microorganisms) on their major histocompatibility complex/human leucocyte antigen (**MHC/HLA**) molecules (pMHC in the figure) that may be recognized by T cells through their T cell receptor (TCR). An additional co-stimulatory signal, usually mediated by CD80/CD86, is required for T cell activation. On the T cell, CD28 acts as a positive co-stimulator, while cytotoxic T lymphocyte antigen-4 (**CTLA-4**) transmits inhibitory (negative) signals upon CD80/CD86 ligation. Polymorphisms in **CTLA-4** that may predispose to AAD may also lead to excessive immune responses in response to certain viruses. Protein tyrosine phosphatase, non-receptor type 22 (**PTPN22**) serves critical roles in the regulation of T cell activation signalling events downstream of the TCR. A specific genetic variant of **PTPN22** may predispose to the activation of self-specific T cells, but also may lead to suboptimal immune responses against influenza. MHC class I polypeptide-related sequence A (**MICA**), a ligand for the co-stimulatory molecule natural killer group 2 member D (NKGD2) on $CD8^+$ T cells, is up-regulated by viral infection. Genetic variants in the transcription factors broad complex-tramtrack-bric a brac and cap'n'collar homology 2 (**BACH2**), nuclear factor of activated T cells (**NFATC1**) and class II major histocompatibility complex transactivator (**CIITA**) have all been associated with AAD. Mutations in **BACH2** may give rise to an immunodeficiency syndrome with recurrent viral pulmonary infections; **NFATC1** plays a role in the process of T cell exhaustion during viral infections, while **CIITA** is a well-known target for immune evasive strategies for several viruses. [Colour figure can be viewed at wileyonlinelibrary.com]

diseases [64]. A common assumption is that autoimmunity risk gene products are involved in the activation and/or regulation of pathogenic or self-specific lymphocytes [65]. However, it is highly likely that these genetic variants also influence the immune responses against microbial agents. A selection of genes associated with AAD that may also play an important role in anti-viral immune responses is shown in Fig. 1. Here we describe how variants in these genes may interact with immune responses against viruses.

AAD is a classic example of a human leucocyte antigen (HLA)-associated organ-specific autoimmune disease [6,8,66]. Similar to type 1 diabetes and coeliac disease, the strongest associations are with the HLA-DR3-DQ2 and HLA-DR4-DQ8 haplotypes [11]. In particular, these two haplotypes in heterozygous combination confer a considerable stronger risk of developing AAD [6]. The high incidence of type 1 diabetes and coeliac disease, and relatively speaking also AAD, in Scandinavia may be the result of the HLA haplotypes HLA-DR3-DQ2 and HLA-DR4-DQ8 being common, because they protected people from succumbing to common infections [67]. Indeed, heritability of susceptibility to infectious diseases is an important functional aspect of HLA class II alleles [68]. For several millennia, in the absence of vaccinations, people survived on the sheer ability to mount protective immune responses to common virus infections, particularly those affecting children [67]. Therefore, the better HLA class II presentation of viral peptides to T cells, and subsequent T cell and antibody responses to measles, rubella, mumps, etc., the greater the chance of survival. Unfortunately, the same HLA class II molecules that have been selected evolutionarily based on their protection against common pathogens might also be particularly suited to present peptides from

self-antigens and thereby participate in autoimmunity [69]. The AAD-associated HLA class II subtype DR4 may serve as an example, as this particular allele is associated with clearance of HBV and HCV viral infections [70,71]. It has also been suggested that the ability of $CD4^+$ T cells to produce certain cytokines in response to infections, such as interferon (IFN)-γ and interleukin (IL)-17, is connected to distinct HLA haplotypes [69]. These particular proinflammatory cytokines may be important in clearing infections, but at the same time act in a detrimental way to the infected host tissue and induce significant immunopathology.

Within the HLA locus, a connection to AAD has also been made with the MHC-class I chain-related gene A (MICA) [8,72], which encodes a ligand for the activating natural killer (NK) and T cell co-receptor natural killer group 2 (NKG2D). MICA can be induced on many cell types in response to cellular stress, such as during a virus infection [73]. The binding of MICA to NKG2D triggers cytotoxicity and cytokine production in NK cells and γδT cells [74], but act as a co-stimulator for activation of conventional $CD8^+$ αβT cells [75]. Ligation of NKG2D on an autoreactive $CD8^+$ T cell by MICA on a virus-infected cell in the setting of self-peptide presentation could therefore lead to excessive activation and expansion of pathogenic $CD8^+$ T cells.

A gene commonly associated with autoimmune diseases is the cytotoxic T lymphocyte antigen 4 (CTLA-4), which acts as an inhibitory immunological checkpoint on activated T cells [76]. Specifically, CTLA-4 is expressed upon T cell activation and opposes continuous co-stimulation of T cells through CD28, as it binds to CD80/CD86 on antigen-presenting cells (APCs) with superior affinity [77]. CTLA-4 therefore accumulates at the immunological synapse between T cells and APCs upon T cell activation, and eventually blocks further co-stimulation and abrogates the T cell response. A recent meta-analysis of European AAD cohorts strengthened the link to CTLA-4 single nucleotide polymorphisms (SNPs) [78], hypothesizing that the associated genetic variants may impair CTLA-4 function and thereby lower the threshold for T cell activation, as suggested in autoimmune thyroid diseases [79]. However, these CTLA-4 polymorphisms may also influence anti-viral immune responses and are associated with certain treatment outcomes in viral infections, e.g. clearance of hepatitis C virus (HCV) after treatment with IFN-α and ribavirin [80]. Another SNP associated with AAD is the 1858T allele of the protein tyrosine phosphatase, non-receptor type 22 (PTPN22) [81]. PTPN22 has both enzymatic and adaptive functions, and modulates signalling through both antigen receptors (T and B cells) and innate immune receptors [82]. The SNP associated with AAD and many other autoimmune diseases may be of particular importance to the regulation of T cell responses to weak self-peptide agonists and promotion of type I IFN production in myeloid cells following Toll-like receptor (TLR) stimulation [83,84]. Interestingly, carriers of the 1858T allele also show an increased susceptibility to infections, including influenza [85].

Polymorphisms in the genes encoding transcription factors broad complex-tramtrack-bric a brac and cap'n'collar homology 2 (BACH2), nuclear factor of activated T cells, cytoplasmic 1 (NFATC1) and the class II, major histocompatibility complex, transactivator (CIITA) have been associated with increased risk of developing AAD [66,86–89]. These transcription factors all play crucial roles in immunity. BACH2 is essential for B cell differentiation into plasma cells and for the generation of regulatory T cells [90]. Mutations in BACH2 lead to an immunodeficiency syndrome with autoimmune components and recurrent pulmonary infections with both virus and bacteria [91]. NFATC1 is involved in gene transcription in activated T cells and is a major target of the immunosuppressive drug cyclosporin [92]. NFATC1 also takes part in T cell exhaustion during chronic viral infections and promotes the expression of inhibitory receptors such as programmed cell death protein 1 (PD-1) [93]. CIITA is considered a global regulator for the expression of MHC class II proteins and related molecules (including MHC class I) [93], and repression of CIITA is part of the immune evasion strategy of several viruses such as EBV and varicella zoster virus [94,95].

Taken together, most of the AAD risk genes regulate or participate in anti-viral immune responses, and the sum of these genetic risk variants could therefore modulate the course of a virus infection. Very little is currently known about such actual gene–environment interactions in the pathogenesis of AAD.

Pathogenesis of autoimmune Addison's disease and implications for viral agents

Although the mechanisms by which the cells of the adrenal cortex are killed by the immune system are starting to unravel, the triggering and perpetuating factors of AAD are still almost completely unknown. The histopathological picture in AAD is characterized by lymphocytic infiltration of the adrenal cortex, while the adrenal medulla remains intact [96–98]. During the course of the disease, the integrity of the cortex is gradually lost as steroidogenic cells are progressively destroyed and replaced by fibrous tissue [12,99]. Despite continuous loss of adrenocortical cells, AAD may remain subclinical for long periods before overt disease

develops [12]. In fact, adrenocortical failure may not manifest itself until 90% of the cells are destroyed [100]. Unfortunately, histochemical studies have been restricted to post-mortem analyses due to practical and ethical reasons, and a detailed analysis of the mononuclear infiltrate in AAD is lacking. Conversely, considerable progress on the disease-specific immunological processes at play in AAD has been made in last decade. Autoimmunity-mediated adrenocortical destruction is a gradual process developing over months and even years involving both T cells and B cells. This process is accompanied by 21-hydroxylase (21OH)-autoantibodies that can be detected in patient serum, constituting an early biomarker of this otherwise silent autoimmune process [6,101]. Although 21OH autoantibodies correlate with the degree of adrenal dysfunction in the preclinical phase of AAD, they do not seem to inhibit 21OH activity *in vivo* [102,103]. Instead, autoreactive T cells specific for 21OH peptides are considered to drive the immunopathology of AAD and indeed, both $CD4^+$ and $CD8^+$ T cells that recognize 21OH-peptides and secrete IFN-γ have been identified [104–107]. 21OH-specific $CD8^+$ T cells from AAD have also been shown to be able to kill adrenocortical cells *in vitro* in an antigen-specific and HLA-restricted manner through perforin and granzyme B-mediated cytolysis [105].

A process in which three major cell types, $CD4^+$/$CD8^+$ T cells and B cells, with specificities for the same antigen, are mobilized and recruited to the adrenal cortex has clear parallels to immunity against infections. It may be assumed that the development of AAD is the result of misdirected processes originally meant to be protective. Given the strong genetic associations to HLA class II haplotypes, the highly co-ordinated specific immune responses against 21OH by both $CD4^+$ and $CD8^+$ T cells and B cells probably depend upon the actions of 21OH-specific $CD4^+$ T helper cells. In this context, many functional aspects of $CD8^+$ cytotoxic T cells against viruses have been shown to depend upon the actions of virus-specific $CD4^+$ T cells, including the primary effector response, the generation of memory and the recruitment to sites of infection [108,109]. This process is known as 'licensing', referring to the ability of $CD4^+$ T cells to license cognate effector $CD8^+$ T cell responses. In AAD, 21OH-specific $CD4^+$ T cells may 'license' 21OH-specific $CD8^+$ T cells to become activated and recruited to the adrenal cortex, leading to adrenalitis and adrenal insufficiency. This process may share mechanisms with the processes that have been described to co-ordinate $CD4^+$ and effector T cell responses to viruses.

Hypothetically speaking, certain viruses could play a role as major environmental factors in AAD, either by infecting steroid hormone-producing cells of the adrenal cortex or by affecting the balance of the immune system. Given the highly co-ordinated immunological attack on 21OH in AAD, and the parallels of these immune responses to those against viruses, we currently believe more in the former. Viruses could spread to the adrenal cortex and induce a strong inflammatory response. In individuals who cannot eradicate the virus efficiently it will remain in the adrenal cortex in a slowly replicating form, continuously producing viral nucleic acids and proteins activating the innate and the adaptive immune system and thus driving chronic inflammation and eventually autoimmunity (probably depending on the genetic background of the host). In addition to the autoimmune reaction against cells of the adrenal cortex, anti-viral immune responses could also participate in the immunopathology. Furthermore, the virus itself may cause cellular damage and stress, and may directly interfere with adrenocortical function (e.g. glucocorticoid production) [110]. Other mechanisms may also be involved, including immunological cross-reactions between viral and host proteins, known as molecular mimicry [111]. We are currently investigating this phenomenon in AAD, as the dominating $CD4^+$ restricted T cell epitopes of 21OH have been identified [105]. Interestingly, some of these epitopes seem to be shared between different HLA class II molecules which could, indeed, implicate molecular mimicry. T cell responses against 21OH epitopes are detectable for decades after disease onset, which could indicate continuous stimulation from cross-reactive viral or microbial peptides. However, remaining or even regenerating adrenal tissue expressing 21OH could also be responsible for the consistent restimulation of 21OH-specific T cells. Fig. 2 describes one possible scenario in which viral infections could play a role in the pathogenesis of AAD, based on current knowledge of disease-specific immunological mechanisms. The figure is not meant to be extensive, and for more comprehensive overviews of the pathogenesis underlying AAD we refer to other reviews written by us and others [2,63]. The IFN-induced chemokine C-X-C motif chemokine 10 (CXCL10) has been shown to be elevated in the serum of patients with AAD [52,112–115]. This elevated CXCL10 production is not due to the activation of peripheral leucocytes, as peripheral blood mononuclear cells (PBMCs) from AAD patients actually show diminished production of CXCL10 upon stimulation with IFNs, compared to healthy blood donors [52]. Instead, primary adrenocortical cells as well as adrenocortical cell-lines of different origins have been shown to be able to produce large amounts of CXCL10 upon stimulation with proinflammatory cytokines such as IFN-γ and TNF-α [112]. Adrenocortical cells are also able to express the TLR for double-stranded RNA (dsRNA), TLR-3 [115].

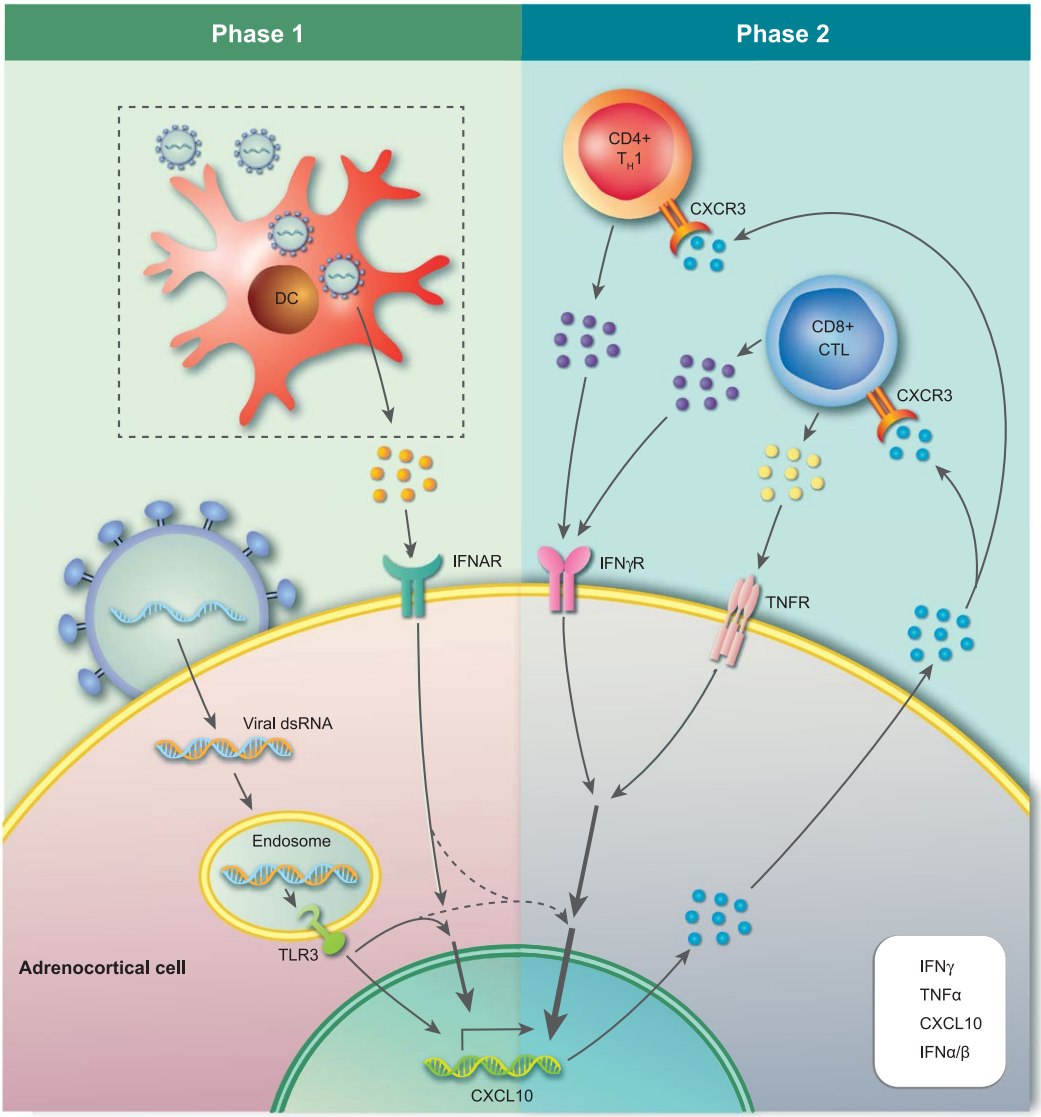

Fig. 2. Hypothetical recruitment of autoreactive T cells to the adrenal cortex triggered by viral infection. Phase 1: a viral infection of the adrenal cortex may serve as the initial trigger of adrenalitis in AAD. Recognition of viral dsRNA by endosomal Toll-like receptor 3 (TLR-3) along with the action of locally produced type I interferons (IFNs) [here by virus-infected dendritic cells (DCs)], stimulates the production of C-X-C motif chemokine 10 (CXCL10) by adrenocortical cells. Phase 2: CXCL10 promotes the recruitment of CXCR3-expressing activated autoreactive $CD4^+$ and $CD8^+$ T cells (e.g. 21OH-specific) that produce proinflammatory cytokines. These T cells are activated and expanded in a local draining lymph node or in secondary lymphoid organs elsewhere in the body (not shown in the figure). This event could be related to the virus infection in phase 1, with DCs and other antigen-presenting cells (APCs) carrying and presenting adrenal components (including 21OH peptides) under proinflammatory conditions, or it could be a result of an earlier infection at a different location than the adrenal cortex (e.g. through molecular mimicry). T cell-derived IFN-γ and tumour necrosis factor (TNF)-α intensify CXCL10 production by adrenocortical cells, leading to a vicious cycle that could strongly expand the mononuclear infiltrate and sustain inflammation of the adrenal cortex. For illustrative reasons, CXCL9 is not included in the figure, but may play an accompanying role in the recruitment of autoaggressive T cells in phase 2. [Colour figure can be viewed at wileyonlinelibrary.com]

Stimulation with the synthetic double-stranded nucleic acid polyinosinic:polycytidylic acid [poly (I:C)], a dsRNA and a ligand for TLR-3, can also induce production of CXCL10, and along with IFN-γ and to a certain degree also TNF-α poly (I:C) is able to drastically increase the CXCL10 production in a synergistic manner. The CXCL10 produced by the adrenal cortex acts as a chemotactic signal for IFN-γ-producing $CD4^+$ and $CD8^+$ T cells bearing the chemokine receptor CXCR3. In concordance with this we have demonstrated that 21OH-specific T cells from AAD patients express high levels of CXCR3 and are able to migrate towards CXCL10-containing cell

culture supernatants from IFN-γ/poly (I:C)-stimulated adrenocortical cells [104]. In a genetically predisposed individual infected by a virus able to infect and replicate in the adrenal cortex, TLR-3 may sense the infection through dsRNA. TLR-3 activation could, in turn, lead to type I IFN production and activation of IFN-stimulated genes (ISGs) such as CXCL10. Alternatively, as the adrenal cortex seems to be a poor producer of type I IFNs [115,116], dendritic cells or macrophages (which are abundantly present in the adrenal cortex) may sense the virus and produce type I IFN. The presence of type I IFNs along with the stimulation of TLR-3 in the adrenal cortex may initiate production of CXCL10, which recruits CXCR3-bearing 21OH-specific T cells from the nearest lymph node. These T cells could, in principle, have arisen years ahead of the ongoing infection, but are only now migrating to the tissue in response to CXCL10 production. Once autoreactive $CD4^+$ and $CD8^+$ T cells arrive in the adrenal cortex, they may become activated through the recognition of cognate HLA-presented 21OH peptides and secrete large amounts of IFN-γ and TNF-α. This could initiate a self-perpetuating inflammatory loop where the adrenal cortex is stimulated to boost the CXCL10 production even further, increasing the influx of proinflammatory CXCR3-positive lymphocytes. The destructive process will continue until the antigen, in this case 21OH, is cleared, which eventually means that the adrenal cortex will be completely atrophied.

Concluding remarks

Unlike other organ-specific autoimmune diseases such as type 1 diabetes, which has been linked to enteroviruses, there are no single obvious viral candidates suggested to take active part in the pathogenesis of AAD. However, the highly targeted and co-ordinated attack by the immune system on the steroidogenic cells of the adrenal cortex, driven primarily by one dominant self-antigen (21OH), is highly reminiscent of that against viruses: $CD4^+$ and $CD8^+$ T cells, as well as antibodies specific for an intracellular antigen. This view is strengthened further by the genetic risk factors associated with AAD, as most of these encode proteins involved in normal anti-viral immune responses. Epidemiological data also point towards a role for infections in the development of AAD. However, the long subclinical phase of AAD makes it difficult to identify any potential viruses that could play a role in the early phases of the pathogenesis. Prospective studies where children are followed from birth to disease onset, such as the TEDDY study for type 1 diabetes [117], are not realistic with a rare disease such as AAD, and as AAD most commonly manifests itself in adults between 20 and 40 years of age. However, if common viruses such as CMV, EBV or HSV-1 are involved in the pathogenesis, several years of prospective studies are a necessity. A possible strategy in the future could be to take advantage of the extremely high heritability of AAD and collect a large cohort of families with aggregation of AAD [14,63]. All family members could then be followed prospectively and monitored for their exposure to infectious agents or other environmental agents, and for any signs of functional adrenal impairment.

Disclosures

The authors declare that they have no conflicts of interest to disclose.

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