Viral infections and autoimmune diabetes

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INFECCIONES VIRALES Y DIABETES AUTOINMUNE

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ABSTRACT
Type 1 diabetes (T1D) is an autoimmune disease that results from the destruction of insulin producing pancreatic islet beta cells. The pathogenesis of T1D is complex and results from a combination of genetic, immunologic and environmental factors. Viruses seem to play a role among the many environmental factors that, together with the genetic susceptibility, have been implicated in the pathogenesis of T1D. Here we review the various hypotheses of the contribution of viruses to autoimmune diseases in general, focusing on T1D: Molecular mimicry, increased processing and presentation of autoantigens during infection or epitope spreading, direct bystander effects, recognition of cryptic epitopes by autoreactive T cells, activation of T cells with dual TCRs, reactivation of memory T cells by nonantigen-specific mechanisms, anti-idiotypic antibodies and superantigens. Epidemiological, serological and experimental studies suggest the association of several viruses to the development of autoimmune diabetes. The most convincing findings are the seasonal variations in the incidence of the disease, the increased frequency of T1D in patients with congenital rubella syndrome or enterovirus infection and the detection of CMV DNA sequences in lymphocytes from T1D patients. However, direct causative effects are difficult to verify. The aim of the present review is to summarize the findings regarding the role of viruses in the development of T1D.

KEY WORDS: Type 1 Diabetes / Autoimmunity / Virus.
INTRODUCTION

Autoreactive T cells and/or autoantibodies cause autoimmune diseases by destroying or functionally impairing targeted tissues. Type 1 diabetes (T1D) is an autoimmune disease that results from the destruction of insulin producing pancreatic islet beta cells. Pancreatic islets are infiltrated by antigen presenting cells and lymphocytes resulting in the destruction of more than 90 percent of the beta cells at the clinical onset of the disease. The incidence of T1D varies depending on the geographical area and is the highest in northern Europe and Sardinia (21 new cases per year for every 100,000 inhabitants), medium in the rest of Europe and USA (4-14 cases) and low in Japan (0.8). The EURODIAB multicentric collaborative study in Europe indicates an annual rate of increase in incidence of T1D of 3-4% with the largest rate of increase seen in children aged 0-4 years(1).

The pathogenesis of T1D is complex and results from a combination of genetic, immunological, hormonal and environmental factors. Genetic factors are considered a major factor in the development of the disease, because there is a strong association between some specific alleles of HLA and other genes and susceptibility to T1D%. Certain HLA haplotypes, such as DQA1*0301-B1*0302 and A1*0501-B1*0201, are positively associated with T1D, whereas others, such as DQA1*0102-B1*0602, are negatively associated with the disease%. Although a genetic predisposition is a prerequisite for developing the disease, concordance rate in identical twins is only about 40%. This fact suggests that non-genetic factors also play an important role in the development of the disease.

Environmental factors such as pathogens, diet, vaccines, stress and toxins are also believed to be involved in the beginning of the autoimmune process. Viruses seem to play a role among the many environmental factors, which together with the genetic susceptibility, have been implicated in the pathogenesis of autoimmune diabetes. Here, we review the various hypotheses of the role of viral infections in autoimmune diseases in general, focusing on T1D.

ROLE OF VIRUSES IN AUTOIMMUNITY:
VARIOUS HYPOTHESES

Microbial infections induce strong specific immune responses, but can also trigger autoimmunity. In this way, it had been proposed that viruses could underlie several autoimmune diseases, such as T1D, multiple sclerosis, autoimmune chronic active hepatitis, Sjögren’s syndrome, juvenile rheumatoid arthritis, thyroid autoimmune diseases and systemic lupus erythematosus. There are numerous proposed mechanisms explaining how viral infections could induce autoimmunity, but the actual mechanisms by which they initiate or precipitate autoimmunity are unknown. These mechanisms involve antigen processing, presentation and activation of the immune system (Fig. 1).

1. Molecular mimicry: microbial antigens share homologies with host antigens

Molecular mimicry has been exhaustively studied as a mechanism of induction of an autoimmune process by a preceding microbial infection and is the most accepted hypothesis. It proposes that viral or bacterial antigens that share homologies with host antigens generate an autoimmune response. The peripheral neuropathy Guillain-Barre syndrome and rheumatic fever are good models of host and bacterial aspects of molecular mimicry resulting in autoimmunity. Also, viruses may encode in their sequence peptides that share homology with self-antigens so T cells and autoantibodies could react with viral and self-peptides during viral infection. This mechanism was first demonstrated by immunization of rabbits with a peptide of hepatitis B virus polymerase in which a six amino acids sequence was identical to the encephalitogenic region of rabbit myelin basic protein (MBP). After viral infection, T cells crossreacted with MBP and the animals developed inflammatory lesions in the central nervous system%. Recent data suggest that cytomegalovirus (CMV) and coxsackie virus could be involved in the
pathogenesis of T1D. Coxsackie B4 virus displays tropism for the pancreatic cells and presents amino-acid sequences similar to that found in the islet autoantigen Glutamic Acid Decarboxylase (GAD). However, a direct demonstration of the implication of molecular mimicry in human autoimmunity is very difficult. Recent studies from animal models demonstrate that molecular mimicry between viral and self antigens can increase but not initiate autoimmunity diabetes. The rat-insulin promoter lymphocytic choriomeningitis virus nucleoprotein (RIP-LCMV-NP) transgenic mouse model of autoimmune diabetes, in which the NP of the LCMV is expressed by beta cells as well as in the thymus, develops autoimmune diabetes after viral infection. Thus, the viral NP acts as an autoantigen but viral infection can increase autoreactive T lymphocytes by cross-reactivity.

2. Increased processing and presentation of autoantigens during infection or epitope spreading

This mechanism involves a continuous acquisition of autoreactive events, leading to a chronic inflammatory state. This activation of multiple autoreactive T cells, as a result of tissue damage, is commonly referred to as epitope spreading and could be related to viral infections, by activating virus-specific T-cells or by direct virus-mediated self-tissue destruction. Virus-specific T cells become activated and migrate to the target tissues where they recognize the viral epitopes. The tissue destruction and release of self-antigens results in a de novo activation of autoreactive T cells and as consequence an autoimmune response. Theiler’s virus model is the experimental animal model for human multiple sclerosis (MS) that has been used to explore this mechanism. Theiler’s murine encephalomyelitis virus (TMEV) is a natural mouse pathogen that persists in the central nervous system. In this model, virus-specific CD4+ T cells initiate the demyelinating process. Epitope spreading and also molecular mimicry are two mechanisms of activation of autoreactive T cells presented in this model. The infection displays a chronic-progressive course with 100% of the animals affected. This mechanism of protection against pathogens resulting in autoimmunity has been postulated for lupus erythematosus, multiple sclerosis and T1D among others.

3. Direct bystander effects: inflammation induced by virus infection

This mechanism consists in the nonspecific activation of autoreactive T cells that have escaped thymic selection in a chronic inflammatory environment induced by viruses. Only a small fraction of activated T cells in viral infections are actually virus-specific. The others proliferate in absence of the first signal (TCR-HLA+peptide). Cytokines, chemokines and other inflammatory mediators are secreted to promote a Th1 response, and increase the expression of MHC molecules, adhesion molecules and costimulatory molecules in the APCs. The altered pattern of expression also affects the target cells i.e. hyperexpression of HLA, adhesion molecules and antigen processing molecules among others. The release of self antigens in the tissue and its presentation by macrophages or dendritic cells may prime virus-specific and autoreactive T cells. This effect has been observed by the transgenic expression of IFN-γ in insulin producing cells or oligodendrocytes resulting in the spontaneous development of diabetes or CNS demyelination respectively. In these experimental models, the upregulation of proinflammatory mediators could cause inflammation, tissue damage, autoantigen spreading, and autoreactive B and T cells activation.

4. Autoreactive T lymphocytes may recognize cryptic epitopes presented during a viral infection

Cryptic epitopes are parts of an antigen that are not processed and not efficiently presented, by contrast to the immunodominant epitope. These epitopes could be sequestered in immunoprivileged organs, presented by inactivated APC, or non normally processed, then inducing self-tolerance. Thus, negative selection allows T cells specific for cryptic epitopes to persist and this process preserves a peripheral repertoire that can participate in antiviral or autoreactive immune responses. In the context of a chronic or acute viral infection, these cryptic epitopes may be efficiently presented by APC contributing to the efficient generation of effector cells and resulting in autoimmunity.

5. T cells with two specificities to viral and self-antigen (Dual TCRs)

It has been shown that certain T cells may express two TCRs of different specificities, due to productive gene rearrangement of both alleles, which makes it a potential risk factor for development of autoimmunity. T cells with specificities for both viral and self-antigen could also respond to self-antigen when activated during viral infection. To date, these mechanisms have been demonstrated in bacterial infections in an experimental mouse model of autoimmune intestinal pathology, mediated by an autoreactive CD8+ T cell clone recognizing epitopes of both mycobacteria and hsp60.

6. Memory T cells and reactivation by nonantigen-specific mechanisms

Autoreactive T cells may be incompletely activated after virus infection outside of the target organ. Reactivation of
these autoreactive resting memory T cells by nonantigen-specific mechanisms –like poly IC or type I interferons secreted during viral infection— may lead to the development of autoimmune disease in the target organ5). This mechanism has been related to relapses observed in MS after a virus infection or vaccination.

7. Anti-idiotypic antibodies

Another theory to link infections and autoimmune diseases is based on the idiotypic network. Antiviral antibodies (first antibody) that arise as a result of viral infection lead to the formation of an anti-idiotypic antibody (second antibody) with autoantibody activity. In this context, several experimental autoimmune diseases were induced in mice following active immunization with pathogenic idiotypes of antibodies. It has been shown that an anti-idiotypic antibody to a monoclonal antibody directed against a rheovirus haemagglutinin, also reacts with rheovirus receptors on lymphocytes and neural cells and some rheumatoid factors appear to be anti-idiotypic antibodies against virus-induced Fc receptor antibodies.

8. Polyclonal activation of T cells by Superantigens

Superantigens are molecules produced by a pathogen that are not MHC restricted and that bind to the specific Vβ chain region resulting in marked polyclonal activation of CD4+ and CD8+ T cells. The ability of superantigens to bind to a wide variety of MHC class II allows the activation of a high number of nonspecific T-cell clones. The best characterized are staphylococcal and streptococcal toxins. Studies using animal models demonstrate that superantigens encoded by viruses, such as mouse mammary tumour virus (MMTV)21, can be implicated in autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), rheumatoid arthritis and psoriasis22, and play an important role in disease progression. In humans, superantigens may play a role in the pathogenesis of autoimmune disorders. It was proposed that type I interferon produced after a viral infection, can induce the expression of a human endogenous retroviral superantigen (IDDMK) resulting in autoimmune diabetes in genetically susceptible individuals23. This mechanism has not been demonstrated in diabetic patients.

VIRUSES AND AUTOIMMUNE DIABETES

Viral infections have been related to the aetiology of TID since the 19th century, when a case of diabetes was reported after mumps infection. The isolation of a virus from the pancreas of a diabetic patient who died at the onset of the disease was reported. Other indirect proofs also support the association between virus and autoimmune diabetes: 1) The seasonal incidence of the disease (autumn and winter) —may lead to the development of autoimmune disease in the target organ. 2) The presence of Coxsackie B, mumps, rubella and cytomegalovirus (CMV) virus-specific IgM antibodies in recent-onset T1D patients; 3) The detection of type I interferons (antiviral cytokines) in the pancreases of recent onset diabetic patients; 4) The presence of enteroviruses RNA in the serum of diabetic patients, and 5) data from experimental studies on animal models.

At present, different viruses have been reported to be associated to T1D in humans and animal models. They might be involved in the pathogenesis of the T1D through release of sequestered antigens by damaged beta cells, by altering some mechanisms of peripheral tolerance, by molecular mimicry, or by a direct destruction of the insulin-producing cells. However, the specific relationship between viral infection and autoimmune diabetes is still in discussion.

Rubella virus

Rubella virus is classified in the Togaviridae family on the basis of its RNA (single stranded, positive sense) genome, icosaedral capsid and lipoprotein envelope. Rubella virus is classified in Rubivirus genus. Rubella is an acute exanthematous viral infection of children and adults. The clinical illness is characterized by rash, fever, and lymphadenopathy. Although many infections are subclinical, this virus has the potential to cause foetal infection with resultant birth defects and, uncommonly in adults, various forms of arthritis.

The congenital rubella syndrome provides the best documentation in humans that viral infections may be associated with the subsequent development of insulin-dependent diabetes mellitus. Patients with congenital rubella syndrome (CRS) have a higher incidence of T1D than the general population. Islet cell and/or anti-insulin autoantibodies have been found in 50 to 80% of diabetic patients with CRS, whereas these antibodies were present in about 20% of non-diabetic patients with CRS. Patients with CRS have a significantly increased frequency of the HLA-DR3 allele (that confers susceptibility to T1D) and a decreased frequency of the protective HLA-DR2 allele.

It has been reported that cytotoxic T cells could recognize rubella virus and GAD65 and 67 by molecular mimicry mechanisms. This finding suggests that in susceptible individuals CRS may generate viral antigen specific cytotoxic T cells either by molecular mimicry or by antigen spreading, finally resulting in beta cell destruction.

Interestingly, neonatal golden Syrian hamsters infected with rubella virus closely parallels the diabetes observed with congenital rubella, showing insulin, presence of...
viral antigens in beta-cells, and cytoplasmic islet cell autoantibodies.

**Enteroviruses**

Enteroviruses (EV) are classified in the Picornavirus family. The sigla of the word «picornavirus» (pico, very small; RNA, nucleic acid type) describe the hallmarks of this large family of animal viruses. Picornviruses are icosahedral and nonenveloped. EV have been divided into several subgroups: i.e. polioviruses, coxsackie B virus (CBV), coxsackie A viruses (CAV) and echoviruses, based on differences in host range and pathogenicity, and further subdivided into more than 70 serotypes. Enteroviruses are found throughout the gastrointestinal tract and, after initial replication in the oropharynx, they can survive in the acidic environment of the stomach and reach the lower intestinal tract, where they replicate more extensively. Epidemiological, serological and biological data suggest that EV could be implicated in the pathogenesis of T1D(41). Antibodies(42) and T-cell responses against enteroviruses have been detected in newly diagnosed T1D patients(43). Thirty three percent of recent-onset T1D patients have IgM antibodies against coxsackie A virus or echovirus(44) indicating a recent infection. Antibodies induced by the capsid protein crossreact with beta cell autoantigen, as IA-2(45). Moreover, a high frequency of enteroviral RNA has been detected in the sera from T1D patients. The variant of coxsackie B4 virus (CVB4) was isolated from the pancreas of a T1D child who died at the onset of the disease and this virus was able to induce diabetes in mice(46).

There are many antigenic variants of CVB4 virus with different tropism. It has been demonstrated that CVB4 virus can infect human beta cells in vitro, decreasing insulin production when the viral titre increases(48). Other results suggest that the quality of the immune response to CVB4 antigens differs significantly between T1D patients and control subjects, with a predominance of primed effector (IFN-gamma-producing) memory cells near to disease diagnosis. This data correlates well with the idea that the diagnosis of T1D is associated with recent or persistent exposure to EV antigens(47). Coxsackie B4 viruses are able to induce the synthesis of interferon-alpha by human beta cells in vitro(48). Furthermore, an increased expression of the interferon-alpha-inducible effector molecule (2’5’ oligoadenylate synthetase) has been detected in diabetic patients(49), reflecting a possible aberrant response to viruses or RNA molecules originating from exogenous or endogenous sources. To date, prospective studies in Northern Europe suggest that enteroviruses may trigger the clinical onset of the disease(50).

**Endogenous retroviruses**

The family of Retroviruses is formed by a large number of icosahedral DNA and RNA reverse transcribing viruses. There was no conclusive evidence for any human retrovirus until 1980 when the isolation of human T-cell lymphotropic virus type I (HTLV-I) from a patient with a T-cell malignancy was reported(51). Most of the mammalian genomes contain non-infectious retroviral sequences, estimated to comprise up to 1% of human DNA. Endogenous retroviruses (ERV) have been implicated in autoimmunity(52), because of their similarities to exogenous retroviruses associated with immune disregulation and their tissue-specific or expression. Retrovirus-like particles and immune responses to ERV proteins have been observed in autoimmune disease although the mechanism by which they are involved in the pathogenesis of the disease is not known. Quantitatively or structurally aberrant expression of normally cryptic ERVs, induced by environmental factors, could initiate autoimmunity through direct or indirect mechanisms. The expression of endogenous retroviruses in insulin producing cells has been associated with insulitis and T1D in NOD mice(53). Retrovirus particles have been detected in the cytoplasm of insulin producing cells in patients with T1D but also in healthy subjects(54). Retroviruses may alter the pattern of expression in the beta cells and the viral antigens may be presented on the beta cell membrane leading to the autoimmune destruction. Moreover, up to 75% of patients with anti-insulin autoantibodies, have also antibodies that recognize both insulin and the retroviral p73 antigen(55). Endogenous retrovirus superantigens could also play a role in the pathogenesis of T1D.

**Reoviruses**

The family Reoviridae includes nine genera of icosahedral, nonenveloped, segmented, double stranded- RNA viruses, four of which cause human disease (orthoreovirus, orbivirus, coltivirus and rotavirus). Reoviruses are associated to diabetes in animal models although their mechanism of action is not known. Viral particles have been detected in the cytoplasm of beta cells in mice, after the induction of the disease by reovirus infection. This suggests that the disease can be caused by a direct effect on the insulin production and secretion of the beta cells(56). Other studies suggest that an autoimmune mechanism might be involved in the development of T1D after reovirus type 1 infection(57). Rotavirus (RV), a genus within the family of reoviridae, may lead to development of diabetes in children genetically at risk because this virus contains sequences highly similar to T-cell epitopes of the islet autoantigens GAD and tyrosine phosphatase IA-2 (IA-2), suggesting a mechanism of molecular mimicry(58).
humans the link between reovirus and diabetes is controversial: a prospective Finnish cohort study was unable to find a relationship between rotavirus infections and T1D in children with genetic susceptibility to T1D(59), whereas another study demonstrated an association between rotavirus infection and islet autoimmunity in children. However, despite human beta cells are susceptible to reovirus infection in vitro(60), to date the involvement of this virus in the pathogenesis of human T1D is controversial.

Mumps virus

Mumps virus is a member of the Paramyxoviridae family, which includes the following genera: Paramyxovirus, Morbillivirus, and Pneumovirus. The complete mumps virion has an irregular spherical shape and the nucleocapsid is enclosed by an envelope that has three layers. The genome of the virus is contained in a nucleocapsid that is a helical structure composed of a continuous linear molecule of single stranded –negative sense– RNA. The capsid protein carries RNA polymerase activity. Mumps is an acute generalized viral infection that occurs primarily in school-age children and adolescents. The most prominent manifestations of the disease are nonsuppurative swelling and tenderness of the salivary glands, with one or both parotid glands involved in most cases. Mumps virus was one of the first viruses associated to the development of human T1D and the mechanism proposed is virus-mediated autoimmunity. Cultured beta cells can be infected with mumps virus in vitro(61). In humans, it has been reported an increase in diabetes incidence 2-4 years after a mumps epidemics(62, 63). T1D patients who had serologically verified recent mumps had more often HLA-DR4 associated risk antigens (Dw4 and Dw14) than other patients. However, an acute or persisting infection in the pancreas at the time of clinical onset of T1D by mumps virus seems unlikely.

Cytomegalovirus

Cytomegalovirus (CMV) is a virus of the Herpesviridae family. The family of Herpesviruses is formed by a large number of icosahedral double-stranded DNA viruses. CMV infection is common in humans and reaches most of the population, whereas associated disease is a relatively exceptional event. CMV shares with other herpesviruses the unique capacity to remain latent in tissues after recovery of the host from an acute infection. Hence, they are opportunists because they are frequently present in hosts waiting to be activated when they become immunosuppressed. Some clinical studies relate human CMV to the development of T1D in humans. The CMV specific viral genome was found in 22% of diabetic patients, correlating with the presence of islet cell autoantibodies in their serum, suggesting that persistent CMV infections may be relevant to pathogenesis in some cases of T1D(64). Molecular mimicry could be involved in CMV-induced diabetes by inducing islet cell autoantibodies(65). In addition, a CD4 T cell clone reactive to GAD65 cross-reacts with a peptide of human CMV major DNA-binding protein(66).

Encephalomyocarditis virus

The encephalomyocarditis virus (ENC) is a member of the Picornaviridae family, which includes both the enteroviruses and rhinoviruses of man as well as the aphthoviruses (foot and mouth disease viruses) and cardioviruses (encephalomyocarditis virus) of hoofed animals and mice, respectively. These viruses are icosahedral and single stranded, positive sense RNA. There are some evidences that diabetogenic variants of encephalomyocarditis virus (EMC) can induce T1D in animal models. It has been shown that the autoimmune destruction of beta cells is dependent on both the genetic background of the host and the genetic variant of the virus(67). Two different models have been established to study the mechanisms of EMC virus induced diabetes. The first one is based on the infection of the mouse with a high dose of EMC virus suggesting that viral replication results in a cytopathic effect and subsequently beta cell destruction. The second model consists in the infection with a low dose of EMC virus. In this model, beta cell infection causes the activation of islet macrophages, the secretion of cytokines and the production of oxygen free radicals thus resulting in the destruction of beta cells(68). Susceptible strains like DBA/1J, when infected with EMC-D—a highly diabetogenic variant- show hyperglycemia and complications similar to those seen in humans with T1D(69).

Kilham rat virus

Kilham rat virus (KRV) is one strain representing the RV serogroup of the Paroviridae family. The Paroviridae are single-stranded DNA viruses. Paroviruses preferentially replicate when cells are in a mitotic stage of division such as is seen during foetal development. Rats are a natural host of KRV and the infection is usually subclinical. KRV was isolated from a spontaneously diabetic rat and reproducibly induced diabetes in naive diabetes-resistant (DR) BB/Wor rats. Viral antigens were not identified in pancreatic islet cells, and beta cell cytolysis was not observed after the appearance of lymphocytic insulitis(70). KRV predominantly infects lymphoid organs and does not infect pancreatic beta cells, indicating that KRV-induced diabetes in DR-BB rats is provoked by an autoimmune process as consequence of a breakdown of the Th1/Th2 immune balance, rather
than by direct cytolysis of beta cells after infection with the virus\(^\text{72}\). Macrophages and cytokines play a critical role in the destruction of pancreatic beta cells in this model\(^\text{72}\). CD4- and CD8+ T cells work synergistically to destroy pancreatic beta cells after KRV infection and the development of the disease is not due to a molecular mimicry mechanism. This model of diabetes may provide insight regarding the interaction of viruses and autoimmune disease.

**Epstein-Barr virus**

Epstein-Barr virus (EBV) is a member of the Herpesviridae family. Virions appear as hexagonal nucleocapsids surrounded by a complex envelope. EBV DNA is double stranded and encodes about 80 proteins. Infection with EBV is common, worldwide in distribution, and largely subclinical in early childhood. EBV has been established as the etiologic agent of heterophile-positive infectious mononucleosis, which occurs most frequently in late adolescence or early adulthood. There is not a strong association between EBV infection and development of T1D. However, some evidence suggests that EBV could trigger TID by a mechanism of molecular mimicry. A sequence in the Asp-57 region of the HLA-DQ chain is repeated 6 times in the EBV-BRF4-encoded epitope. Some individuals who carry this sequence (GPPAA) in their HLA-DQ molecule present antibody reactivity to this epitope in EBV. As reported by Parkkonen F et al., two out of seven individuals who had acute EBV infection produced antibodies against an EBV-derived peptide (GPPAAGPPAAGPPAA). These two cases also contracted T1D immediately after the infection. This phenomenon may have potential importance in EBV-induced abnormalities, although cross-reactivity against DQ molecules could not be demonstrated in this study\(^\text{73}\).

**Mengovirus**

Mengovirus is a member of the genus Cardioviruses of the Picornaviridae family. The genus currently comprises of two virus species: *Encephalomyocarditis virus* (includes encephalomyocarditis virus, mengovirus, and other strains) and *Theilovirus*. These viruses are icosahedral and single stranded, positive sense RNA. Mengovirus can infect and destroy pancreatic beta cells in rodents and sometimes in humans (direct cytolytic infection). In mice, Mengovirus produces a fatal encephalitis. There is a clone of Mengovirus, Mengovirus-2T that in addition to encephalitis causes diabetes in mice, producing beta cell necrosis and severe infiltration\(^\text{78}\), Viral antigens were found in the islets. Studies on susceptibility among inbred strains of mice showed that whereas the D variant of encephalomyocarditis virus caused diabetes only in SJL/J mice, Mengo-2T caused diabetes in several strains of mice resistant to D variant (i.e., CBA/J, C3H/HeJ, CE/J, AKR/J, C57BL/6J). The ability of Mengo-2T to induce diabetes in encephalomyocarditis-resistant mice was found to be due to the greater capacity of Mengo-2T as compared to the Mengo-D variant to replicate in beta cells and destroy the islets of these animals. Although Mengo-2T and the D variant of encephalomyocarditis virus are antigenically indistinguishable by hyperimmune sera, these studies show that these viruses have different host ranges and tissue tropisms.

**Bovine viral diarrhoea-mucosal disease virus**

Bovine viral diarrhoea-mucosal disease virus (BVDV) is a pestivirus of the Flaviviridae family (single stranded, positive sense RNA viruses) and is antigenically related to hog cholera virus and border disease virus of sheep. Although cattle are the primary host for BVDV, the virus infects most even-toed ungulates. BVD-MD has been reported to be associated with T1D in cattle, although not all animals infected with this virus develop diabetes\(^\text{79}\). The differences between individuals could be linked to different variants of the virus or to genetic susceptibility differences among the hosts.

**Ljungan Virus**

Ljungan Virus is a new species of Picornaviridae family (single stranded, positive sense RNA viruses) isolated from bank voles. Comparisons with other picornaviruses indicate that the capsid-coding genes of Ljungan Virus are most closely related to human parechovirus types 1 and 2, members of the newly created picornavirus genus *Parechovirus*. It has been described that 33% of wild bank voles, when kept in captivity, develop diabetes\(^\text{76}\), with autoantibodies to GAD65, IA-2 and insulin. The islets of these animals stained positively for this virus. Infecting wild bank voles with the virus can induce experimental diabetes.

**Possible role of other viruses**

Furthermore, some circumstantial evidences point out the relationship between other viruses and the development of T1D in humans: hepatitis A virus\(^\text{77}\), varicella zoster virus\(^\text{78}\), measles virus, polio virus and influenza virus\(^\text{79}\). However, further investigation is needed to find the relationship between these viruses and the development of T1D.

**OTHER EVIDENCE FROM ANIMAL MODELS**

It is very difficult to link viruses and T1D in humans. Animal models of T1D, spontaneous, induced or genetically modified, have been developed to test the hypothesis of
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Virus-induced diabetes and the mechanisms of action. Transgene technology has been used to examine the possible roles of virus-related molecules, by fusing the regulatory elements of the rat insulin gene (rat insulin promoter, RIP) with the gene of interest. This technique has provided important information on the consequences of islet expression of viral proteins and antiviral cytokines in normal mice and in the autoimmune diabetes-prone NOD mice. The expression of a viral transgene in the beta cells was not associated with T1D. However, when the host becomes infected with a virus encoding the same gene as the transgene, the immune response against the virus also recognizes the transgene resulting in autoimmunity to insulin producing cells. This process depends on activated autoreactive T cells, costimulatory and accessory molecules and cytokines produced. The transgenic expression of type I interferons (IFN), a product of many cells infected by virus, resulted in insulitis and T1D in non-diabetes prone mice(81-84) and in an acceleration of the onset of the disease in NOD mice(85). This fact suggests that an initial stress (possible a viral infection) may induce type I IFN by the islets and induce autoimmunity. By contrast, it is well known that the development of T1D in humans and animal models is reduced in the presence of high amounts of pathogens. Viruses can cause but also prevent autoimmune disease: the exposure of NOD mice to Mouse Hepatitis Virus (MHV) decreases cumulative incidence of diabetes. Virally induced cytokines and chemokines can influence the progression of autoimmunity and depending on the time, location and level of expression, can prevent the disease. These transgenic models allow dissecting the role of virus, self-proteins and immune responses in the complex process of autoimmunity towards beta cells. The timing of the infection, the viral strain, the antigenic load, and other unknown factors are crucial in the protection or predisposition to the disease.

CONCLUSIONS

At present, several viruses have been reported to be associated with the development of autoimmune diabetes in both humans and animal models. The evidences are indirect and came from epidemiological, serological and experimental studies. Environmental factors seem to be decisive in the development of autoimmune disease in genetically susceptible individuals. Seasonal variations in the incidence of the disease, with peaks in autumn and winter, and the existence of peaks at the age when children start school suggested that common viruses could be involved in the pathogenesis of T1D. It is difficult to study the role of viruses in the development of human diabetes due to the anatomical inaccessibility of the pancreas and the long term between viral infection and clinical symptoms. To date there are controversial results about the implication of viruses in human islet autoimmunity. The most convincing findings are the increased frequency of T1D in patients with congenital rubella syndrome, enterovirus infection and the detection of CMV DNA sequences in lymphocytes from T1D patients.

Viral infections can result in an enhancement of the MHC class I expression in the beta cells, thus inducing an enhanced display of self-peptides favouring autoimmunity. This enhancement may occur by a direct interaction between the viral component and the MHC-class I gene or indirectly by virus-induced soluble factors such as type I IFN in the beta cells. In spite of that, direct causative effects have been difficult to verify, and islet autoantibodies may already be present in cord blood from children who later develop T1D, suggesting that the islet autoreactivity developed before viral infection. Further knowledge of the relationship between viruses and autoimmunity may help the design of more accurate prevention strategies.

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