Viral infections in the pathogenesis of autoimmune diseases: focus on type 1 diabetes

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. The case for viral-induction of type 1 diabetes
4. Viruses associated with the induction of type 1 diabetes
   4.1. Mumps virus
   4.2. Rubella virus
   4.3. Enteroviruses
   4.4. Rotavirus
   4.5. Cytomegalovirus
   4.6. Endogenous retroviruses
   4.7. Parvoviruses
   4.8. Other viruses
5. Mechanisms of induction of type 1 diabetes by viral infections
   5.1. Direct destruction of pancreatic beta cells
   5.2. Molecular mimicry
   5.3. Bystander damage (release of sequestered antigens)
   5.4. Disruption of regulatory T cell and effector T cell balance
   5.5. Superantigens
   5.6. Multiple infections
6. Concluding Remarks
7. Acknowledgements
8. References

1. ABSTRACT

Susceptibility to autoimmune diseases is dictated by a complex interplay of genetic determinants and environmental factors. Viral infections have long been suspected to be involved in the etiology of several autoimmune disorders. In particular, the incidence of type 1 diabetes worldwide is increasing at a yearly rate that cannot be solely attributed to genetic changes in the population and environmental factors certainly play an important role in the pathology of this autoimmune disease. In this review, we will focus our discussion on the evidence supporting a role for viral infections in the pathology of type 1 diabetes. We will place a particular emphasis on the major candidate viruses and on the current state of knowledge regarding the mechanisms responsible for the induction of autoimmunity following viral infections. The lessons learned from type 1 diabetes provide a great framework of knowledge that can be applied to most viral-induced autoimmune diseases.

2. INTRODUCTION

Autoimmunity results from a loss of tolerance leading to an inappropriate immune response directed against self antigens leading to organ-specific destruction or systemic disease. The predisposition of an individual to autoimmune diseases is largely, but incompletely, dictated by genetic determinants. Several lines of evidence suggest a significant contribution from environmental factors in both the induction and the progression of autoimmune disease in genetically predisposed patients. Among these environmental factors, viral infections have long been suspected as agents in the pathology of autoimmune diseases. Clinical and epidemiological evidence has linked a plethora of viruses to organ specific autoimmune disorders including Type 1 diabetes (T1D) (1), autoimmune myocarditis (2) and multiple sclerosis (3) as well as several systemic autoimmune disorders including, systemic lupus erythematosus (4) and myasthenia gravis (5) (Table 1). However, despite several years of research, it has remained
Viral infections in the pathogenesis of autoimmune diseases

Table 1. Viruses associated with the induction of autoimmune diseases other than T1D

<table>
<thead>
<tr>
<th>Autoimmune Disease</th>
<th>Associated Viruses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune Myocarditis</td>
<td>Coxsackievirus</td>
<td>148</td>
</tr>
<tr>
<td>Autoimmune Thyroiditis</td>
<td>Epstein-Barr virus</td>
<td>149</td>
</tr>
<tr>
<td>Human T-Lymphotropic virus type 1</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>Coronavirus</td>
<td>152, 153</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>154-156</td>
<td></td>
</tr>
<tr>
<td>Human herpes virus 6</td>
<td>157</td>
<td></td>
</tr>
<tr>
<td>JC virus</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>Measles virus</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>Endogenous Retroviruses</td>
<td>160-162</td>
<td></td>
</tr>
<tr>
<td>Myasthenia Gravis</td>
<td>Hepatitis C Virus</td>
<td>163</td>
</tr>
<tr>
<td>Human foamy virus</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>Human T-lymphotropic virus type 1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>Epstein-Barr virus</td>
<td>165</td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>Coxsackievirus</td>
<td>167</td>
</tr>
<tr>
<td>Epstein-Barr</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>169</td>
<td></td>
</tr>
<tr>
<td>Human herpes virus 6</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Human T-Lymphotropic virus type 1</td>
<td>171</td>
<td></td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Cytomegalovirus</td>
<td>172</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>174</td>
<td></td>
</tr>
</tbody>
</table>

Difficult to directly establish a causal link between specific viruses and the induction of autoimmunity in humans. This is most likely explained by the complex interplay of genetic and environmental factors that lead to disease induction following infection. This suggests that the majority of individuals infected with a particular virus harboring the capacity to induce autoimmune diseases do not develop autoimmunity, thereby rendering the determination of a single culprit difficult. Furthermore, establishment of disease will often occur long after viral clearance making it difficult to properly identify any potential causative agents. Despite these difficulties, research in small animal models has firmly demonstrated that under certain circumstances viral infection can induce or exacerbate autoimmunity defining an important role for viruses in the etiology of autoimmune disorders. In this review, we will focus on the role of viruses in the induction of T1D as this has been the subject of intense research over the last few decades. We will discuss both the epidemiological data linking several viral candidates to disease onset and the various mechanisms of disease induction that have been proposed. Although this review focuses on T1D, the mechanisms discussed here are likely to extend to the induction of several other autoimmune disorders following viral infection.

3. THE CASE FOR VIRAL-INDUCTION OF TYPE 1 DIABETES

T1D results from the destruction of the insulin producing beta cells in the islets of Langerhans of the pancreas by autoreactive T cells. Disease onset occurs after a large majority of beta cells have been destroyed and the autoimmune response is initiated years before appearance of clinical symptoms (6). Progression of the autoimmune response can be, in part, followed by measuring levels of beta cell antigen specific autoantibodies which appear long before the onset of clinical symptoms (7). If left untreated, T1D can lead to several life threatening symptoms and the only treatments currently available involve the direct injection of insulin or islet transplantation (8). Several factors seem to combine to confer susceptibility to T1D with genetic determinants playing a prominent role. The human leukocyte antigen (HLA) region is the primary genetic determinant of susceptibility (9) and several other genetic loci, including the insulin promoter (10), PTPn22 (11) and CTLA-4 (12-14), also combine to increase susceptibility. Several genetic regions contribute to confer susceptibility to diabetes development in the non-obese diabetic (NOD) mouse, a commonly studied animal model of T1D, where spontaneous development is controlled by at least 20 genetic loci including the major histocompatibility region (15). In humans, the rate of diabetes is increasing worldwide at a rapid rate (16-19) that cannot be explained by changes in the genetic makeup of the population alone particularly as the incidence of disease has been on the rise in populations with HLA alleles generally associated with reduced susceptibility (20). T1D often presents with geographic distribution patterns that do not correlate with genetic differences. For example, there is a large discrepancy in the incidence of T1D between Finland and neighboring regions of Russia despite similar prevalence of HLA alleles associated with susceptibility to disease (21). Furthermore, although certain populations are associated with lower incidences of T1D, immigration to areas associated with higher risk correlates with an increase of T1D within these normally protected populations. For example, although Asian populations have one of the lowest T1D incidences in the world, prevalence of T1D in Asian populations living the United Kingdom is almost identical to the higher prevalence that occurs within the caucasian population (22). Most convincingly, the concordance rate of T1D incidence in monozygotic twins is approximately only 40% clearly indicating that environmental factors combine with genetic predisposition to influence the likelihood of developing disease (23). These factors are likely to include changes in diet, exposure to beta cell toxins or pathogens such as viruses (24).

Viruses were first recognized as inducing agents of T1D when it was observed that disease onset sometimes follows acute viral infections. This was further supported
with epidemiological data indicating that T1D onset follows seasonality in both hemispheres in a manner that is strongly correlated with the seasonality of a number of viral infections (25, 26). Several instances of local epidemics of T1D resembling sudden infectious epidemics have also been reported (27-30). Furthermore, seasonal onset is often observed in less genetically predisposed populations, suggesting the contribution of risk factors other than genetic determinants in those cases (31). This may also explain why broad human studies, which are usually carried out on the most genetically susceptible populations, have commonly failed to clearly identify any candidate viruses. Most convincingly, two separate groups have reported the isolation of enteroviruses directly from the pancreas of patients that succumbed to acute onset T1D. Upon adaptation of these strains to allow them to replicate in mice, infection with these isolated viral strain induced hyperglycemia and a diabetes-like disease in infected animals, clearly demonstrating that viruses can lead to T1D (32, 33). One of the strains isolated from an acute onset T1D patient was identified as coxsackievirus B4 (CB4) (32) and has become a commonly used tool to study the induction of T1D by viruses. The diabetogenic properties of CB4 have since been clearly demonstrated in genetically susceptible mouse models (34-36). Accumulating evidence points to viral infections representing at least one of the environmental stimuli responsible for the rapid increase of T1D observed worldwide in the last few decades.

4. VIRUSES ASSOCIATED WITH THE INDUCTION OF TYPE 1 DIABETES

Since the first reports of T1D onset following a viral infection were published, a large number of viruses have been suggested as potential triggers of autoimmune diabetes. In this section, we will discuss the most likely candidates for the induction of T1D and the data supporting a role for each of these viruses in the induction of disease.

4.1. Mumps virus

Mumps is a single-stranded RNA virus from the paramyxovirus family and was one of the first viruses linked with diabetes. In the late 1800’s, a case of acute onset diabetes was reported in a patient shortly following a mumps infection (37). This causal link was later supported by the observation that mumps epidemics were sometimes followed by sudden sharp increases in T1D onset a few years later (38). Mumps virus infections are further correlated with the presence of islet specific antibodies (39). Since the early 1980’s, vaccination campaigns aimed at controlling measles, mumps and rubella (MMR vaccine) have been in place in most western countries. One follow-up study in Scandinavia reported a plateau in the rising incidence of T1D a few years following the start of this vaccination campaign (40), strongly suggesting a direct role for one of these viruses in the etiology of T1D. However, despite the ongoing vaccination efforts, the plateau in T1D incidence was short-lived and incidence is once again rising sharply (16-19). This implies that although one of the targets of the MMR vaccine may have been a major environmental contributor to T1D, other viruses are also involved. This further suggests that vaccination approaches aimed at reducing diabetogenic infection in children may represent a fruitful strategy to reduce T1D onset.

4.2. Rubella Virus

Rubella is a single stranded RNA virus from the togavirus family and has been strongly linked to T1D. Approximately 20% of congenital rubella syndrome (CRS) sufferers have been reported to develop T1D later in life (41-46). Interestingly, some studies have failed to demonstrate the presence of increased beta cell specific antibodies in CRS patients that develop T1D suggesting that congenital rubella-induced T1D may not be autoimmune in nature but may instead result from direct effects of the viral infection (47). However, evidence from animal models (48) and the presence of cross-reactive determinants between rubella virus and GAD65 (49), a pancreatic beta cell autoantigen, still suggest that autoimmune processes may be involved. Although CRS has clearly been linked to the induction of T1D, vaccination campaigns have dramatically reduced Rubella virus infections worldwide without affecting the increasing incidence of T1D onset. This suggests that Rubella virus infections are unlikely to be the main etiological agent responsible for the current rise of T1D.

4.3. Enteroviruses

The enterovirus genus of the picornaviridae family includes several common human pathogens. Enteroviral infections in humans typically occur through the oral-fecal route and once infection is established, the virus can disseminate to a number of organs including the pancreas (50, 51). Members of this genus, particularly coxsackieviruses and echoviruses have long been suspected to be environmental inducers of T1D. The first evidence of a link between coxsackieviral infection and autoimmune diabetes was presented in a report by Gamble et al. in which a higher prevalence of viral-specific antibodies in T1D patients compared to control populations was demonstrated (52). This was later supported by the isolation of coxsackievirus directly from the pancreas of a child that had died as a result of acute onset of T1D (32). Infection with this isolated virus was subsequently observed to induce a diabetes-like disease in mice (32). The same viral strain was further demonstrated to possess the capacity to induce or dramatically accelerate disease onset in two mouse models on the NOD genetic background (34, 35). Several studies have since confirmed the presence of increased coxsackievirus specific antibody titers in recent onset patients compared to control populations (52-58). Furthermore, recent onset T1D patients present with higher prevalence of enteroviral RNA compared to control patients (59, 60). A recent report detailed an active echovirus 3 infection concurrently with the development of IA-2 and islet cell autoantibodies (61). Interestingly, it was recently demonstrated that recent-onset T1D patients have decreased T cell responses in vitro to CB4 antigens. This suggests that patients at risk for T1D may have difficulty clearing coxsackieviral infections potentially leading to heightened pancreatic damage and thereby increasing the likelihood of autoimmune consequences (62). Conversely, it was demonstrated that patients with ongoing diabetes have a greater frequency of CB4 specific T cells than recent
onset or non-diabetic patients inferring that coxsackieviral infections may also exacerbate pre-established autoimmunity (63). Congenital enterovirus infections have also been linked to the pathogenesis of T1D as one group reported that patients that developed T1D tested positive for neonatal enterovirus RNA more commonly than a control population that did not develop disease (64). Taken together, this evidence strongly suggests that enteroviruses are good candidates as one of the major environmental agent contributing to the increased T1D incidence observed over the last few decades.

Despite these strong indications of the role of enteroviruses in the pathogenicity of T1D, results from several large epidemiological studies have remained inconsistent. The Finnish diabetes prediction and prevention study demonstrated an important temporal relationship between enteroviral infection and development of T1D with infections detected in 57% of children that developed T1D associated autoantibodies within a 6 month period compared to only 31% in age-matched control children that did not develop autoantibodies (65). A separate study in Finland confirmed that the presence of enteroviral RNA was elevated in children that develop autoantibodies as compared to children from a control population (66). Interestingly, viral RNA was not detected in diabetic children demonstrating that, although the presence of viral RNA in children that have not yet developed the disease could be considered a risk factor for T1D, viral persistence probably does not play a role in the autoimmune pathology (67). These results were further confirmed in the Trial to Reduce IDDM in the Genetically at Risk project where a greater percentage of children that developed autoantibodies were positive for both enteroviral RNA and antibodies compared to age matched children that do not develop autoimmunity (68). These results, however, have been contradicted by several other studies. For example, it was determined that overall levels of enterovirus infections are lower in countries with high incidence of T1D as compared to countries with higher frequencies (e.g.: Finland and Russia) (69). Several reports from longitudinal studies in Europe (70), the United States (71) or Australia (72) have further failed to demonstrate a link between any enteroviral infections and the development of T1D related autoantibodies. Several factors could explain these discrepancies. It is important to consider that these studies use different methodologies and the populations studied have different genetic profiles rendering direct comparison difficult. Further, there is a large number of enterovirus serotypes (73) and it is possible that not all of these will demonstrate diabetogenic properties possibly confounding the analysis of some of the data. Finally, these studies have all been interested in demonstrating a link between enteroviral infection and the onset of autoimmunity. However, as will be discussed below (section 5.3.), results from animal models indicate that coxsackieviral infection may simply precipitate ongoing autoimmune processes rather than initiating them. Taken together, the accumulated data strongly suggests a link between enteroviruses and T1D and the controversy that remains simply highlights the need for continued research in this area.

4.4. Rotavirus

Rotavirus is a double stranded RNA virus from the reovirus family that is a major cause of gastroenteritis in children worldwide (74). Rotavirus replication is usually focused in gut epithelial cells but it also has been demonstrated to cause acute systemic viremia and some cases of pancreatitis have been documented (75, 76). They were first suspected to be involved in the etiology of T1D due to shared sequences between a viral protein (VP7) and some major T cell epitopes from antigens derived from pancreatic beta cells (77). Furthermore, infections with rotavirus occur predominantly in young children (74), an age group that has seen a dramatic rise in incidence of T1D in the last decades. Longitudinal studies in Australian children at risk of T1D have demonstrated increased incidence of islet specific antibodies shortly after detection of rotavirus infection (72). This study further demonstrated that increases in islet antibodies were correlated with signs of repeated rotavirus infection indicating that yearly epidemics of this virus could contribute in an additive fashion to the induction of islet specific autoimmunity (72). These results are further supported by studies demonstrating that reoviruses can specifically infect pancreatic beta cells and induce diabetes in infant mice (78, 79). The plausibility of the role of rotavirus in human T1D was further enhanced by the recent discovery of T cells specific for the shared VP7 sequences in children presenting with signs of autoimmunity strongly suggesting the presence of cross-reactive responses between rotavirus and pancreatic beta cells (47). However, a second study of children at risk in Finland did not validate these results and failed to associate rotavirus infection with the development of islet specific antibody (80). These studies, however, only used IgG levels to define rotavirus infection and used a viral strain uncommonly found in humans for their analyses, two factors that may have led to an increased frequency of false negatives and render this study more difficult to properly analyze (47, 80). These differences in methodology leading to contradicting results highlight some of the difficulties inherently associated with conclusively determining the role of any particular pathogens in the induction of autoimmunity in human populations. Despite these contradictions, it seems likely that rotavirus may also contribute to the pathogenesis of T1D and together with enteroviruses may be responsible for the increased incidence of T1D in certain parts of the world.

4.5. Cytomegalovirus

Cytomegalovirus (CMV) is a member of the herpes virus family and has been associated with induction of T1D by a few studies. The link between CMV infection and T1D induction was first established in a report of a child developing T1D after suffering from a congenital CMV infection (81). This was later supported by a report of a patient with CMV infection developing T1D after suffering from severe pancreatitis (82). Studies of non-diabetic siblings of T1D patients have demonstrated a strong correlation between anti-CMV antibodies and the presence of islet-specific antibodies (83). Another study demonstrated that 80% of patients with islet specific antibodies tested positive for both anti-CMV antibodies and
4.6. Endogenous retroviruses

Endogenous retroviruses are commonly found within mammalian genomes. They represent proviral DNA from retroviruses that have become associated with host following integration within the genome. An endogenous retrovirus named IDDMK1,222 was originally isolated from the pancreas of two recent onset T1D patients and was believed to be responsible for the induction of T1D (92). It was hypothesized that a virally encoded superantigen may have been responsible for inducing or expanding self-reactive T cells in a non-specific manner leading to the targeting and destruction of the pancreas in genetically susceptible individuals (see section 5.3.) (92). However, this retrovirus was later demonstrated to be ubiquitous within human populations and not specifically associated with T1D patient casting a doubt over its role in autoimmunity (93, 94). Other lines of evidence still link endogenous retroviruses to T1D. For example, 64% of sera from diabetic patients and 75% of sera from non-diabetic first-degree relatives of T1D patients presented with anti-insulin autoantibodies that cross-reacted with a retroviral antigen compared to only 3% in age-matched controls (95). Another study observed retrovirus-like particles specifically in the pancreas of patients who died shortly after T1D onset while none were observed in control patients (96). These data are further supported by research in the NOD mouse model where expression of endogenous retroviruses has been associated with the start of invasive insulitis and the onset of T1D (97-99). In that model it has been hypothesized that the presentation of retroviral antigens following a wave of beta cell apoptosis that is a potential inducer of onset of autoimmunity in the NOD mouse and could represent a key step in the induction of autoimmunity (96). Alternatively, it remains possible that the onset of autoimmunity is associated with the expression of endogenous retroviruses and that expression of these viruses is a result rather than a cause of autoimmunity. The role of endogenous retroviruses in the induction of T1D remains very intriguing and further research on the subject is needed to further clarify their potential role.

4.7. Parvoviruses

There is very little data linking parvoviral infection to the onset of T1D in humans. One report described the onset of several autoimmune phenotypes including T1D in one patient following acute parvovirus B19 infection (100). Conversely, another study of recent onset T1D patients failed to establish a link between disease onset and parvovirus B19 infection (101). This study, however, focused solely on recent onset patient which cannot rule out the role of parvovirus infections prior to onset of disease as a modifier of susceptibility to T1D. Despite the paucity of information linking parvovirus B19 to T1D in humans, one of the most described animal models of T1D induction by viruses relies on the Kilham Rat virus (KRV), a member of the parvovirus family. KRV infections are sufficient to induce T1D in diabetes resistant Bio-Breeding rats (BBDR), a BBDP derived rat strain that does not spontaneously develop diabetes (102). Interestingly, KRV does not directly infect the islets (103) but, rather, induces T1D by affecting the delicate balance that maintains peripheral tolerance (see mechanism discussion below, section 5.4.) (104, 105). Further studies, possibly taking advantage of the longitudinal studies of children at risk, are needed to clarify the role of parvovirus infection in human T1D.

4.8. Other viruses

Several other viruses including Epstein-Barr virus (106, 107), Hepatitis A virus (108) and Hepatitis C virus (109) have been associated with onset of T1D. Although in most cases, the data associated with these viruses remains sparse or inconclusive indicating that further research is required. The large number of viruses that have been associated with the induction of T1D highlights that viral-induction of T1D may represent a common final step associated with several infections or even combinations of different infections. Taking into account that most human studies have a limited pool of participants to draw from, it is easier to understand why it has remained very difficult to conclusively identify any single viral culprit in the induction of autoimmune diseases.

5. MECHANISMS OF INDUCTION OF TYPE 1 DIABETES BY VIRAL INFECTION

The mechanisms through which viruses ultimately lead to autoimmune diseases such as T1D in humans are poorly understood. Several hypotheses have been proposed but very little human data is currently available to support any of them. Research in animal models has advanced our understanding of how some of these mechanisms relate to induction of disease. One key highlight from this research is that a wide variety of mechanisms could be used by viruses to induce autoimmunity and that this is also likely to be true for humans. Understanding the exact mechanisms by which viruses can induce autoimmunity may allow a better evaluation of the epidemiological data with a view towards finding new candidate viruses. More importantly, this may lead to the design of new therapies aimed specifically at reducing the autoimmune consequences of certain infections. In this section we will discuss the data
Viruses can also cause autoimmune diabetes through molecular mimicry, where the virus has antigens that are similar to those of the β cells. This similarity can lead to the immune system attacking the β cells as if they were part of the virus, leading to diabetes.

5.1. Direct destruction of pancreatic β cells

One of the simplest mechanisms through which viruses can induce T1D in patients is by direct infection and destruction of the insulin producing β cells in a process that does not involve any autoimmune reaction. The picornavirus encephalomyocarditis virus (EMCV) has been demonstrated to induce T1D in animal models. Infection with a high dose of the D strain of EMCV results in acute onset of T1D in more than 90% of infected animals within 4 days of infection (110). Treatment of mice with antibodies that block T cell responses were not sufficient to block induction of T1D following infection indicating that destruction of the β cells is not mediated by T cells (111). Evidence does point to a partial contribution for macrophages in the destruction induced by EMCV but the exact mechanism remains to be elucidated (112). Infections of mice with lower doses of EMCV viruses that are more likely to represent a natural infection also resulted in induction of diabetes and this induction was entirely dependent on macrophages, potentially through the release of cytokines and free oxygen radicals (112-114). Another member of the picornavirus family, the cardiotropic Mengovirus, also results in direct destruction of beta cells in mice and the induction of T1D without any evidence of an autoimmune response (115). There is very little evidence to link either of these viruses to diabetes induction in humans. The extremely rapid onset of diabetes in mice following infection is more reminiscent of the few cases of “fulminant” T1D that have been reported in humans (116). As such, this mechanism is unlikely to be responsible for the steady increase of autoimmune diabetes worldwide, where disease induction is usually preceded by a long period of asymptomatic autoimmunity.

5.2. Molecular mimicry

Molecular mimicry is an elegant theory that stipulates that regions of similarities between viral and self antigen epitopes could lead to an inappropriate cross-reactive immune response resulting in autoreactive T cell activation and tissue destruction. This theory was first formulated when it was observed that monoclonal antibodies raised against viral protein could cross-react with cellular epitopes (117). Molecular mimicry has been proposed to explain the viral etiology of several autoimmune diseases including multiple sclerosis (118) and T1D (119). As mentioned in the previous sections, several potential mimics between β cell antigens and T1D associated viruses have been identified including CB4 (120, 121), rotavirus (77) and CMV (85). In the late 1980s, two separate models were established by the groups of Dr. Oldstone and Dr. Zinkernagel to test the potential role of molecular mimicry in the viral induction of T1D (122, 123). In both of these models, a viral protein of the lymphocytic choriomeningitis virus (LCMV) was expressed directly within pancreatic β cells under the control of a rat insulin promoter (RIP-LCMV). Peripheral tolerance to these neo-antigens was maintained and no spontaneous diabetes was observed in these models. However, LCMV infection resulted in rapid induction of diabetes in almost all of the mice compared to no diabetes induction following LCMV infection of non-transgenic littermates (122, 123). Although these models elegantly demonstrated that molecular mimicry could, in principle, be responsible for the induction of T1D following infection, these models rely entirely on identity (the protein expressed in the pancreas is identical to the one expressed by the virus) and, therefore, do not represent a situation that would naturally be encountered. These models and others relying on the pancreatic expression of neo-antigens derived from a virus have also been extremely useful to further dissect some of the requirements of viral-induction of autoimmunity. First, they demonstrated that the mere presence of self-reactive T cells is not sufficient to induce autoimmunity. For example, it was demonstrated that several lines of LCMV-GP mice (transgenic mice expressing the glycoprotein of LCMV within the pancreas) expressed the neo-antigen exclusively in the beta cells and this was correlated with very high numbers of autoreactive T cells compared to transgenic mouse lines where the expression of the antigen was also detectable in the thymus (124). Despite these high numbers of autoreactive T cells T1D did not develop spontaneously confirming that changes induced following infection are still required for induction of disease (124). These models have further highlighted the role of cytokines and chemokines as blocking the effects of IFN-gamma, TNF-alpha or IP-10 by genetic or antibody blocking methods have all been demonstrated to either abrogate or dramatically reduce T1D onset following infection (125-127). These results indicated that in the presence of a high number of self-reactive T cells, changes in the cytokine/chemokine milieu induced by viral infection can be sufficient to precipitate the induction of autoimmunity. The RIP-LCMV model was also used to demonstrate that a true mimic can at least accelerate autoimmunity although, in that model, it was not sufficient to induce autoimmunity by itself. A cross-reactive epitope between the nucleoprotein of LCMV and Pichinde virus (PV) has been described. This cross-reactive section occurs over a stretch of 8 amino acids, of which 6 are identical, on a sub-dominant epitope that does not play a major role in initial infections with either virus (128). Although PV infection was not sufficient to induce T1D in RIP-LCMV mice, it was observed that a PV infection 4 weeks after initial LCMV infection can dramatically enhance onset of disease (129). These results highlight that once autoimmunity process has begun, infection with a virus that bears a cross-reactive mimic might result in disease enhancement. Therefore, it is possible that in patients where autoimmune attack of the pancreas is already ongoing, infection with any virus that includes cross-reactive epitopes may serve to precipitate destruction of islet cells and onset of disease. This mechanism could therefore extend to a variety of the candidate virus that have been discussed in this review but proper timing of infection would be required for these viruses to induce disease. Alternatively, molecular mimicry may be an important source of potentially autoreactive T cells. Peripheral tolerance could be maintained until subsequent events alter the regulatory T cells (Tregs)/effector T cell balance. As such, molecular mimicry between one virus and pancreatic self-antigen can serve to modify pre-
Viral infections in the pathogenesis of autoimmune diseases

disposition to disease as well as precipitate ongoing autoimmune processes.

5.3. Bystander damage (release of sequestered antigens)

Several T cell receptor transgenic models were generated on the NOD background to study the role of T cell clones of various specificities in the progression of T1D. One of these, the BDC2.5 model, was created from a T cell clone of unknown specificity derived from a diabetic NOD mouse (130, 131). Interestingly, despite the majority of T cells in this model being specific for a pancreatic antigen, these mice never develop diabetes or progress beyond a stage of non-invasive insulitis (termed peri-insulitis) where T cells home to the pancreas without infiltrating or destroying the beta cells (34, 132). This model further demonstrates that the mere presence of autoreactive T cells is not sufficient to induce disease. Infection of these mice with coxsackievirus B4 was sufficient to rapidly induce T1D in the majority of infected mice (34). Interestingly, the gender bias normally observed for the spontaneous induction of T1D in NOD mice is not observed following infection of BDC2.5 mice strongly suggesting that the induction of disease following infection involves mechanisms that are distinct from the mechanisms of spontaneous disease induction (34). Although the exact specificity of the BDC2.5 clone remains unknown, it was determined that the clone does not directly respond to CB4 antigens. This strongly indicated that, despite regions of shared specificity between the viral P2-C protein and the pancreatic antigen GAD65, molecular mimicry is not responsible for induction of disease following CB4 infection, at least in the BDC2.5 model (34). Instead, CB4 infection induced T1D by causing bystander damage leading to the release of previously sequestered pancreatic antigens leading to the activation of the pre-existing self-reactive T cell population (34). This further suggests that ignorance of an autoreactive T cell clone’s cognate antigen is an important mechanism for the maintenance of peripheral tolerance to pancreatic islet antigens. CB4 infects pancreatic beta cells without direct induction of cell death (133). Instead, infection leads to the upregulation of cell surface markers associated with cellular stress, particularly Death Receptor 4 and subsequent engulfment of infected islets by antigen presenting cells (APCs) (133). Adoptive transfer of purified APCs from CB4 infected NODscid mice were later demonstrated to be sufficient to induce BDC2.5. T cell proliferation in vitro and, more importantly to adoptively transfer disease to uninfected BDC2.5. mice clearly confirming the importance of APCs in the induction of T1D following infection (133). Our recent results have demonstrated that transgenic expression of TGF-beta within pancreatic beta cells, protects from CB4-induced T1D and that this correlated with a decreased activation of macrophages but not dendritic cells (Richer et al., manuscript in preparation). Our results further highlight the importance of APCs, particularly macrophages, in the induction of T1D following CB4 infection. The role of APCs has been well described in spontaneous T1D (134) and it seems likely, based on the literature, that they would also play a central role in the induction of T1D following CB4 infection. Studies on the BDC2.5 model have further supported that the primary contribution of CB4 infection to the induction of disease is to cause pancreatic damage and release sequestered antigens. Treatment with the double-stranded RNA molecular mimic, poly I:C, was not sufficient to induce disease in BDC2.5 mice (135). Poly I:C is recognized by receptor Toll-like receptor 3, an innate receptor involved in viral recognition, leading to a cascade of events culminating in the production of pro-inflammatory cytokines. These results strongly suggest that non-specific activation of T cells mediated by viral-induced inflammatory cytokine is not sufficient to induce disease. Conversely, it was demonstrated that a single low dose of streptozotocin was sufficient to induce disease (135). At low dose, this pancreatic toxin results in minimal pancreatic damage and was demonstrated to partially mimic the effects of CB4 infection by causing the engulfment of beta cells by APCs and activation of the pre-existing population of autoreactive T cells (135). The presence of a pre-existing population of autoreactive T cells seems to be an absolute requirement for CB4-induction of T1D based on data gathered in two separate mouse models. BDC2.5 mice were crossed with two separate mouse strains resulting in crossed strains presenting with a varying level of autoreactive T cells. Only mice harboring a high (WT BDC2.5 mice) or intermediate (BDC2.5 X Balb/C F1 mice) level of autoreactive T cells were susceptible to viral induced T1D while mice with low levels (BDC2.5 X C57Bl/6 F1 mice) of autoreactive T cells were protected from disease induction (136). In the NOD mouse, it was observed that only mice that were at least 8 weeks old were susceptible to acceleration of disease following CB4 infection while younger mice were impervious to disease (35). The authors suggested that the accumulation of a critical threshold of autoreactive T cells was a strict requirement for induction of disease. These results were later confirmed for other diabetogenic viral strains of coxsackievirus where only older NOD mice were susceptible to viral-induced acceleration of disease (137). Taken together, this indicates that coxsackievirus infection represents that last step in disease induction and essentially serves to precipitate the already ongoing autoimmune reaction. This mechanism is likely to extend to any virus that can induce stress or cause damage to the pancreatic beta cells.

5.4. Disruption of regulatory T cell and effector T cell balance

Tregs are a specialized subset of T cells involved in maintaining peripheral tolerance and have been ascribed an important role in the prevention of several autoimmune diseases (138). Tregs from diabetic patients have significantly reduced suppressive function as compared to non-diabetic control subjects (139) while in NOD mice, Tregs gradually lose their suppressive capacity and this gradual loss correlates with onset of disease (140). Other T1D models derived from the NOD mouse such as BDC2.5. and NOR mice, do not spontaneously develop disease unless Tregs are depleted (141, 142). These results clearly highlight the important role of Tregs in the progression of T1D. Any disturbances affecting the finely tuned balance between autoreactive T cells and Tregs could potentially lead to or dramatically exacerbate autoimmunity. Interplay between Tregs and virus has been
shown to play a role during infections (143). Increased Treg presence can be associated with viral persistence and this, in turn, could lead to increased tissue damage thereby increasing the likelihood of autoimmunity (143). Conversely, viral infections could either directly or indirectly reduce Treg function or numbers thereby releasing autoreactive T cells from suppression leading to increased autoimmunity. To this effect, it has been demonstrated that one of the cytokines commonly produced during an acute response to viral infection, interleukin-6, is an important factor to release effector T cells from Treg suppression thereby allowing for the development of an immune response (144). Clearly, any infections leading to prolonged release of interleukin-6 could result in the inappropriate release of autoreactive T cells and promote autoimmunity. KRV infections of BBDR rats induce diabetes in this normally resistant rat model without direct infection of islet cells (103). This has been hypothesized to be a result of changes in the effector T cell/Treg balance following viral infection. Infection of BBDR rats with KRV led to an upregulation of Th1-like CD45RC⁺ CD4⁺ cells and cytotoxic CD8⁺ T cells and a downregulation of Th2-like CD45RC⁻CD4⁺ cells (104). This indicated that alteration in the balance of T cell polarity following infection was sufficient to induce disease. It was later demonstrated that KRV infection of BBDR mice also affects the Treg balance. Following infection the levels of CD4⁺CD25⁺ T cells were decreased in the spleen of BBDR rats compared to mice infected with a closely related virus that does not induce T1D. Surprisingly, the presence of CD4⁺CD25⁺ T cells in the pancreatic lymph node was increased (105). The authors concluded that these changes in Treg populations were responsible for induction of disease although it may be important to revisit these experiments now that reagents that allow a more specific characterization of Tregs are available. In the same rat model, it has been demonstrated that activation of innate cells with TLR ligands synergizes with viral infection to induce a higher incidence of disease (145). These results further highlight that the fine balance of activation of T cells versus the maintenance of tolerance is crucial to the decision for progression towards autoimmunity. The modulation of Treg responses following infection are likely to be involved in the autoimmune pathology observed following several viral infections and should be the focus of more investigation.

5.5. Superantigens

Superantigens are gene products encoded by some bacteria and viruses that lead to the non-specific expansion of T cells bearing a particular Vbeta region on their T cell receptor. Superantigens were first linked to T1D when it was observed that T cells infiltrating the pancreas of two diabetic patients were dramatically enriched for the Vbeta 7 variable region (146). This possibility was further supported by the isolation of an endogenous retrovirus, from diabetic patients, that was found to encode a superantigen (92). Although the link between this particular endogenous retrovirus and T1D has since been questioned (93), the role of superantigens in T1D remains intriguing. Infection with any virus encoding for a superantigen could lead to the non-specific expansion of diabetogenic T cell clones thereby leading to disease onset. Alternatively, increased presence of diabetogenic T cells may not directly lead to disease but may simply predispose the host to induction of disease following a viral infection that leads to bystander damage thereby precipitating disease onset.

5.6. Multiple infections

Research by the groups of Dr. Selin and Dr. Welsh has established that sequential infections have dramatic effects in shaping T cell repertoires (147). Furthermore, different combinations or sequences of infection can lead to the generation of memory T cells with varying capacity to cross-react with other sequences depending on the order of infection (147). As such, a person’s history of infection can influence immunity following every new infection and may have a strong effect on the development of immune pathology. Therefore, multiple infections are likely to represent a risk factor for T1D. For example, a primary infection could generate autoreactive T cells either through molecular mimicry or by influencing the cross-reactivity of memory T cells. Further infections can then induce disease either by expanding these autoreactive T cells above a certain threshold or by inducing bystander damage leading to the release of previously sequestered antigens recognized by these autoreactive T cells. It is therefore possible to envision that T1D induced by viral infection is in fact the result of a combination a various infections using a variety of mechanisms ultimately leading to the destruction of the pancreatic beta cells. This may, in part, explain why it has remained so difficult to identify any single culprit virus as a clear inducer of T1D.

6. CONCLUDING REMARKS

A strong causal link in humans supported by research in animal models has demonstrated that viruses can induce or accelerate the onset of autoimmune diseases including T1D. Despite this strong link, it has remained difficult to conclusively identify any single virus due to a variety of factors that have been described throughout this review. It is becoming clear that the mechanisms through which can viruses can induce T1D and likely other autoimmune disease are complex and varied. It seems increasingly likely that T1D induction by environmental agents does not occur by a single mode of disease induction but rather a complex interaction which may include several viruses, alone or in combination, leading to disease through several mechanisms. The early results that demonstrated that MMR vaccination led to a plateau in T1D incidence in certain countries gives hope that managing infections in young children may prove an efficient way to control the rising incidence of autoimmune diseases (40). To reach this goal however it will be imperative to maintain research aimed at identifying potential viral culprits, understanding the mechanisms of induction of disease following infection but also research aimed at better understanding the biology of some of these viral infections in order to be able to design vaccines or more efficient therapies aimed at controlling infections that can potentially lead to autoimmune consequences.
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Viral infections in the pathogenesis of autoimmune diseases


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