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Review

Diabetes mellitus due to viruses – some recent developments

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Summary. Many different viruses belonging to several genera have the potential to damage beta cells. The mechanisms they employ are varied, and infection may result in either a direct destruction of islets and rapid insulin deficiency, or in a more gradual loss of functioning islets with the onset of diabetes many years later. Several case histories involving extensive cytolysis of beta cells can be directly linked to viral infection, whilst an example of diabetes occurring many years after viral infection is found in individuals who had a congenital infection with rubella virus. Here, the virus induces an autoimmune reaction against beta cells. Autoimmune phenomena have also been observed in islets following infections with viruses other than rubella, and thus activation of autoimmune mechanisms leading to beta-cell destruction may be a relatively frequent occurrence. Recent evidence shows that picornaviruses are not exclusively lytic, and can induce more subtle, long-term changes in beta cells, which may be important in the aetiology of diabetes. The exact mechanisms involved are not known, but it is clear that several viruses can directly inhibit insulin synthesis and induce the expression of other proteins such as interferons, and the HLA antigens. Strain differences in viruses are important since not all variants are tropic for the beta cells. Several laboratories are in the process of identifying the genetic determinants of tropism and diabetogenicity, especially amongst the Coxsackie B (CB) virus group. The sequence of one such diabetogenic CB4 strain virus has been determined. It is clear therefore that there are many viruses with the potential to induce diabetes, and a viral involvement in the pathogenesis of diabetes has been established in some instances. Further research work at both a fundamental and epidemiological level is now urgently needed to define the nature of the interaction of such viruses with the beta cell.

Key words: Beta cell, Coxsackievirus, diabetes, insulin-dependent diabetes mellitus, islet, picornavirus, virus.

Infections with many unrelated viruses have been associated with the development of diabetes mellitus, and almost one hundred years ago an observation that diabetes followed a recent infection with mumps virus was recorded [1]. In recent years a clearer relationship between a number of viral infections and the onset of diabetes has become better established, though a final picture of the relevance of viruses to diabetes and in particular to insulin-dependent diabetes, has yet to emerge. A review of recent developments with particular reference to the picornaviruses seems timely. This is especially so, since there appears to be a greatly renewed interest in the importance of environmental factors in the genesis of diabetes [2, 3]. Viruses are the most likely of such factors to be involved in the development of insulin-dependent diabetes. This view is based on many serological and epidemiological studies [4–8] and case histories [9–11]. As shown in Table 1, the link between picornaviral infection and diabetes appears to be particularly strong and the two most notable case histories of viral-induced insulin-dependent diabetes involved members of the Coxsackie B (CB) group (Table 2). It has also been noted that the incidence of diabetes and glucose intolerance has increased following epidemics of picornavirus infection [12–14]. Moreover recent genetic studies detailing the sequence of some diabetogenic and pancreotropic strains of picornaviruses have been carried out [15–17].

There are two possible ways in which a virus could produce diabetes. The first is when overt disease develops after an interval of many years from the time of infection with virus. This is exemplified by diabetes in the congenital rubella syndrome (CRS) where infection takes place in utero and diabetes occurs 5–20 years later [18]. The second way appears to involve a direct assault on the pancreatic islets during the course of an acute viral infection. The full expression of diabetes resulting from a direct attack alone is probably a rare

Table 1. Picornaviruses which have been implicated as causative agents of diabetes in humans and in animals

Genus	Virus	Effect
Aphthovirus	Foot and mouth disease virus	Diabetogenic in cattle [101]
Cardiovirus	Encephalomyocarditis virus (myocardial strain)	Diabetogenic in mice [63]
	Mengovirus (clone 2T)	Diabetogenic in mice [102]
Enterovirus	Poliovirus	Diabetes reported in man following infection [103]
	Coxsackievirus (A group)	Raised IgM levels in diabetic patients [104]
	Coxsackievirus (B group) (several serotypes)	Diabetogenic in humans and in mice [10, 53, 69], in vitro effects on beta cells [66, 67]
	Echo virus (4)	Effects on human and mouse beta cells [12, 70]
	(2)	Raised IgM levels in diabetic patients [104]

Table 2. Case studies in which picornaviruses have been incriminated in the onset of diabetes in man

Virus	Reference	Observations
Untyped Coxsackie B	Sussman et al. 1959 [105]	Islet degeneration, no inflammation
Coxsackie B1/B5	Nelson et al. 1977 [106]	Raised antibody titre, Bornholm disease
Coxsackie B1	Kaplan et al. 1983 [107]	Infection in utero, insulitis, virus in pancreas
Coxsackie B2	Wilson et al. 1977 [108]	Islet lymphocytosis
Coxsackie B4	Gladisch et al. 1976 [9]	Insulitis, viral antigen in islets
Coxsackie B4	Yoon et al. 1979 [10]	Lymphocytic infiltration, virus isolated from pancreas diabetogenic in mice
Coxsackie B4	Asplin et al. 1982 [109]	Islet cell antibodies
Coxsackie B4	Nihalani et al. 1982 [110]	Generalised Coxsackie B4 infection
Coxsackie B5	Champsaur et al. 1980 [11]	Virus isolated from stools produced glucose intolerance in mice
Coxsackie B5	Ahmad and Abraham 1982 [111]	Mononuclear infiltrate in islets
Coxsackie B6	Nigro et al. 1986 [87]	Islet cell antibodies

event. However, limited attacks by beta-cell tropic viruses leading to temporary hyperglycaemia or permanent subclinical damage to the islets may well occur. As is becoming evident this type of viral insult could easily be the triggering event for a complex series of immunological changes which are associated with beta-cell destruction.

Apart from mumps and rubella virus, and some picornaviruses, there is recent evidence of possible associations of diabetes with cytomegalovirus (CMV) and retroviral infection. The majority of CMV infections, like rubella, are acquired congenitally and are initially subclinical. However, diabetes as a complication of CMV infection has been recorded [19]. One study has shown that about 15% of newly-diagnosed insulin-dependent patients have CMV-specific viral genome in their lymphocytes and islet cell antibodies (ICA) in their sera [20], indicating that autoimmune diabetes is sometimes associated with persistent CMV infection. Human CMV can also induce an ICA which reacts with the 38 kDa autoantigen isolated from human pancreatic islets [21].

The possible importance of endogenous retroviruses in the development of diabetes has been brought to light by studies of the non-obese diabetic (NOD) mouse and the Biobreeding rat. In these models, the development of autoimmune diabetes appears to be conditional upon the presence of retroviruses. Clusters of retroviral particles (A type) have been found in beta cells of NOD mice in association with the insulitis preceding diabetes [22]. The exact role of retroviruses in the disease process is not clear, but specific expression of endogenous retrovirus by beta cell is linked to the development of insulitis and diabetes in these mice.

In addition in some mouse strains, treatment with streptozotocin induces aberrant retrovirus (C type) budding into the rough endoplasmic reticulum of beta cells before insulitis [23, 24]. Intracisternal type A particles have also been observed in the necrotic beta cells of genetically diabetic mice [25].

Picornaviruses and induction of diabetes

Picornaviruses associated with either human or animal diabetes are shown in Table 1. Of these only the CB viruses and possibly those of the echo group appear to be linked to the development of diabetes in man. Epidemiological and serological data which support this idea have been discussed in considerable detail elsewhere [26, 27]. Data on the appearance of Coxsackie-specific antibodies following diabetes are controversial, and difficulties are encountered with their interpretation. The majority of studies however, have found higher levels of viral antibodies in newly-diagnosed diabetic patients than in control subjects. Some studies have reported a lower viral antibody titre in diabetic children [28] indicating that there may be a reduction in immunological competence in individuals genetically susceptible to diabetes, or that exposure to virus in these individuals is less and results in a more severe disease later. Generally IgM levels are high during the early stages of an infection and their presence signifies an infection having occurred within the previous 8 weeks, which would suggest that an acute rather than persistent infection precedes the onset of diabetes. In one study, circulating

IgA antibodies are present in individuals several years post-infection and a higher prevalence and mean titre of these CB antibodies have been found in diabetic children compared to control subjects [29], suggesting perhaps the establishment of a persistent infection.

The strongest evidence implicating members of the CB group as causative agents of insulin-dependent diabetes comes from the study of a number of case histories (Table 2). The most important of these involved CB4 and CB5 viruses [10, 11]. In both instances virus was isolated from the patients (CB4 specifically from the pancreas) and produced diabetes or glucose intolerance, respectively in susceptible mice. It is clear therefore, that in at least some instances the onset of diabetes is directly related to recent CB infection.

Viral tropism

The diversity of symptoms occurring in picornaviral infection are indicative of the fact that the picornaviruses are composed of many virions with distinct tropisms for specific tissues. Within a clinical isolate there are variants with different biological properties. The phenotypically stable diabetogenic encephalomyocarditis virus (EMC) contains a mixture of diabetogenic and non-diabetogenic virions [30]. CB viruses predominantly attack only the acinar tissue, but occasionally damaged islets have been observed [31, 32]. There are instances in which direct associations between CB viruses and diabetes exist, but since diabetes is only sometimes a sequel to outbreaks of CB virus infection, this indicates that only some variants of virus circulating in the natural population are tropic for the beta cells. Coleman et al. [33] used an unadapted CB4 isolate to infect mouse islets in vivo and in vitro and a transient hyperglycaemia developed in diabetes-susceptible mice. Other studies using clinical isolates similarly found that a proportion of mice develop hyperglycaemia or abnormal glucose indices [34, 35]. Furthermore a significant number of random clinical isolates impaired islet function in vitro [36], and it is possible they could inflict subclinical damage in vivo, which in some individuals could contribute to the eventual decline and destruction of beta cells.

Tropism (the characteristics of a virus to infect a specific tissue or cell type) is thought to be regulated by attachment of virus to receptors. The expression of receptors is genetically determined and influenced by cell maturation and its functional state. As might be expected the passage of CB viruses through beta cells results in an increased tropism for these cells, by a selection of those viral variants that attach and preferentially replicate in them. This has been demonstrated in several studies [37, 38]. In EMC infection of diabetes-susceptible mice it has been found that viral titres are higher and the number of infected cells is greater than in non-susceptible mice due to a greater replication in the former. Similarly evidence exists for a greater replication of a mouse pancreas-adapted CB4 virus in murine islets compared to an unadapted CB4 virus (T. Szopa, unpublished data).

Persistent infections, mutations, and islet tropic strains of virus

Both host and virus selective pressures operate during an infection. The virus undergoes both phenotypic and genotypic changes, and these become particularly important during the course of a persistent infection. The picornaviruses were originally described as highly lytic viruses but it is now known that they can also establish persistent infections, and can cause damage by other means than a simple lysis. There is evidence for the persistence of poliovirus [39] and echo virus 9 and 30 in man [40]. The CB viruses have also been found to persist in man [41] and in mice [42, 43]. In vitro several cell types e. g. lymphocytes, fibroblasts and pancreatic tumour cell lines can be infected in this manner by CB viruses [44–46] and also by echo 6 virus [47]. Particularly interesting are the reports of CB infection of human lymphoid cell lines and persistent CB4 infection of rat insulinoma cells in the absence of cytopathology [44–46]. A latent CB4 infection of a rat insulinoma cell line has been reported in which a change in cell function occurred with little change in morphology [48]. Significantly an acinar-tropic variant of CB4 has been found to persist in the pancreas of mice for several months post-infection [49] and it is possible than from this type of virus a highly beta-cell tropic variant could arise which would then attack the islets, some time after the initial infection.

There are two mechanisms by which RNA viruses can persist in cells – the steady-state or the carrier-culture system. In a steady-state infection all the cells are infected, whilst in the carrier culture only a proportion of cells is involved. The most likely mechanism thought to be used by the picornaviruses is the carrier-culture system, although a few members of the group e. g. echo 6 virus have been shown to establish steady-state infections [47].

Persistent infections are more readily established in infants whose immune system is immature e. g. rubella virus infection in utero which leads to autoimmune diabetes [50] by an as yet unknown mechanism. Similarly studies of CMV infection in utero have shown that there are islet cell antibodies (ICA) in newly-diagnosed diabetic patients and human-specific viral genome in lymphocytes [20, 21]. Diabetes in this case could result from a response to expression of viral antigens or induction of beta-cell specific autoantigens.

The rate of mutation in picornaviruses is high [51] and accounts for the variety of clinical manifestations of infection. In CB4 virus the rate is estimated to be 10^{-4} mutations per base [52]. This high frequency means that diabetogenic variants could arise spontaneously in nature, or that during the course of either a subclinical infection or persistent infection a virus could at some time mutate into a strongly beta-cell tropic variant. Evidence that beta-cell tropic variants exist quite commonly in CB4 infection has been presented [36].

Long-term defects in islet function were found to occur in mice after infection with a mouse pancreas-adapted strain of CB4 virus 6 months earlier [53]. Although the islets of these mice were histologically normal and gross changes in blood glucose level were not found, insulin se-

Table 3. Nucleotide differences between the prototype strain of coxsackie B4 virus (JVB) and the diabetogenic strain

Nucleotide position[a]	CB4 JVB	P-CB4 Diabetogenic	Codon change	Amino acid change	Viral protein
131	T	C			
136	T	A			
137	A	G/C			
171	T	C			
546	G	C			
572	T	C			
750	G	A	GCA-ACA	Ala-Thr	VP4
812	G	A	TCG-TCA		
1112	C	T	ACC-ACT		
1646	T	C	GTT-GTC		
1787	T	C	GAT-GAC		
2480	G	A	ATG-ATA	Met-Ile	VP1
3296	C	T	CCC-CCT		
4307	C	T	TAC-TAT		
4988	G	A	AGG-AGA		
5015	G	A	GCG-GAG		
5086	T	C	GTA-GCA	Val-Ala	P3A
5124	A	G	ATT-GTT	Ile-Val	P3A
5147	G	T	AAG-AAT	Lys-Asn	P3A
5176	T	C	ATT-ACT	Ile-Thr	P3A
5405	T	C	AGT-AGC		
5541	T	C	TTA-CTA		
5594	G	A	CGG-CGA		
6084	T	C	TTC-CTC	Phe-Leu	P3D
6275	A	G	TTA-TTG		

[a] Jenkins et al. 1987 [57]

cretion and synthesis in the islets was impaired. How these changes induced by the virus persist is not known at the present time but the observations suggest that CB viruses are capable of inducing subtle changes in islet cell metabolism which may be important in the pathogenesis of insulin-dependent diabetes.

Amongst other virus groups, a certain strain of lymphocytic choriomeningitis virus (LCMV) causes a persistent infection of beta cells in mice and induces changes in function without destruction of the cells [54]. This could occur by a reduction in the number of beta cells following infection, since the regenerative capacity of beta cells is poor, or alternatively a differentiated cell function (e.g. insulin production) could be turned off.

The genetic basis of viral diabetogenicity

The genetic basis of viral diabetogenicity can be investigated by comparative nucleotide sequence analysis of viral genomes. The complete nucleotide sequences of the diabetogenic EMC (D strain) and the non-diabetogenic EMC (B strain) have been established [55, 56] and it has been concluded that there are 14 nucleotide differences between the two strains. A second non-diabetogenic EMC variant has been sequenced in order to focus on the nucleotide changes responsible for the diabetogenic phenotype [15]. It appears that only two amino acids may be responsible for the diabetogenicity of EMC, one on the leader peptide and one on the coat protein VP1.

A similar approach has been used to examine the diabetogenic phenotype of CB4 virus. The complete nucleotide sequence of a mouse pancreas adapted variant of CB4 has been determined [16] and compared to that of the previously published sequence of the non-diabetogenic prototype CB4 strain (JVB Benschoten) [57]. Twenty-five nucleotide sequence differences have been identified (Table 3). Of these differences, six occur in the 5' non-coding region of the genome and 19 in the coding region, resulting in seven amino acid changes. These comprise one in each of the coat proteins, (VP1 and VP4), one in the polymerase gene (P3D) and four in another non-structural protein (P3A), for which a clear role has not yet been defined.

It can only be speculated upon at this time as to which of the nucleotide changes contribute to, or are responsible for, the diabetogenic phenotype in CB4, particularly as phenotypic changes may involve one or a few genomic alterations. Conversely, multiple mutations or deletions may be required. Whilst nucleotide changes were found in the coat proteins of the pancreas-adapted CB4 (in VP1 and VP4) and in the processing proteins (in P3A and P3D), it is the changes in the 5' non-coding region that are of most interest. The involvement of picornaviral 5' non-coding regions in viral replication has been and still is the subject of intense study. Alignment of enteroviral 5' non-coding region sequences highlights several stretches of conservation and pyrimidine rich regions with the potential to form stem and loop structures [58]. These common features suggest a functionally important role for the 5' non-coding region. Indeed for poliovirus some of the structural elements within this region have been shown to be necessary for efficient translation and replication of the viral genome [59–62]. The nucleotide changes identified in the genome of the beta-cell tropic CB4 5' non-coding region occur in the regions corresponding to those de-

scribed for poliovirus. Whether the 5' non-coding region nucleotide changes act to alter the rate of viral replication alone, or in combination with nucleotide changes occurring elsewhere in the genome warrants further investigation.

Metabolic effects of picornaviruses on islets

Studies in whole animals

Extensive literature now exists on these effects. The earliest studies were with EMC virus where the myocardial variant induced very marked elevations of blood glucose in diabetes-susceptible mice [63]. In later studies CB4 and CB5 virus was isolated from patients dying in ketoacidosis and when injected into susceptible mice produced hyperglycaemia or glucose-intolerance respectively [10, 11].

In general however, the CB viruses are not strongly tropic for the beta cells and it is only in rare instances that a completely unadapted human CB isolate produces hyperglycaemia in mice [33]. This does not mean that more subtle changes might be induced in islets, as is explained later. Serial passage of CB1–CB6 through beta-cell cultures resulted in an increased tropism for beta cells and transient hyperglycaemia developed in mice following infection with them [38]. Similarly passage of CB4 through monkey cell cultures produced a virus capable of damaging islet function resulting in abnormal glucose tolerance [64]. The effects were enhanced by previous streptozotocin treatment indicating that viral infection may cause significant damage when acting upon a pancreas with a depleted cell reserve.

Studies in isolated islets

A number of studies show that picornaviruses may attack the islets directly causing impaired insulin synthesis and release [65, 66]. In isolated mouse islets infected with a mouse pancreas-adapted CB4 preparation, over a 4-day period, insulin synthesis was reduced and insulin release at basal glucose concentration was increased [66]. Similar results were obtained when human islets were infected with a non-adapted CB4 preparation in vitro [67]. CB3 infection of human islets in culture also resulted in a decreased intracellular insulin content which appeared to parallel the increase in virus titre [68].

Mice infected with variants of CB4 tropic for beta cells show impaired function in islets for up to 6 months post infection [53]. Higher than normal amounts of insulin were released at non-stimulatory glucose concentration while glucose-stimulated insulin synthesis was inhibited. There was no evidence of changes in blood glucose in these animals or of histological damage to islets. Another group, using a CB4 variant plaque-purified from a myocardial isolate, found that beta cells from infected mice produced less insulin and that total protein synthesis was also reduced [69]. These changes related to alterations in blood glucose levels.

Similar experiments have been carried out in vitro and in vivo with mouse islets infected with a variant of echo 4 virus [70]. This variant was derived from an isolate obtained from an outbreak of echo 4 virus-induced meningoencephalitis among children in Cuba. It has also been reported that a significant number of these children developed glucose intolerance and ICA after infection [12].

Mechanism of effects of diabetogenic viruses on protein synthesis in islets

Much attention has been directed to the effects of viruses and particularly the picornaviruses on protein synthesis in islets. These viruses may affect insulin biosynthesis or the synthesis of other islet proteins such as the HLA antigens or the interferons. In several instances diabetogenic picornaviruses appear to be able to reduce insulin biosynthesis. This can sometimes take place without any concomitant destruction of beta cells and therefore it may be quite unrelated to cytolytic destruction as was first thought to be the case.

Insulin biosynthesis

It was shown in 1975, that EMC virus reduced proinsulin synthesis in mouse islets [71]. The mechanism by which viruses reduce insulin synthesis is of great interest. With EMC virus the effect is biphasic. Insulin biosynthesis has been shown to be diminished as early as 2-h post infection in dispersed islets [72]. In whole islets there is no change in insulin secretion this early. In the second phase the virus produces a progressive loss of preproinsulin mRNA. This effect appears to be selective since mRNA for the constitutive protein glyceraldehyde 3-phosphate dehydrogenase was not reduced until long after infection. The early effects appear to be due to a "shut-off" of host protein synthesis as has been described for virus effects in other host tissues.

Some similar effects on insulin synthesis have been observed in mouse islets infected with a beta-cell tropic strain of CB4 [73, 74]. Again at an early phase of viral activity there is a selective inhibition of insulin synthesis.

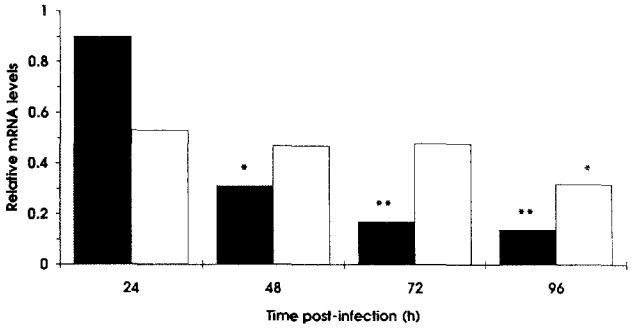

Fig. 1. Preproinsulin mRNA (■) and glyceraldehyde 3-phosphate dehydrogenase mRNA levels (□) in coxsackie B4 virus – infected mouse islets. Significant differences from the 24 h levels are denoted by * $p < 0.002$ and ** $p < 0.001$

This is again accompanied by a rapid depression of preproinsulin mRNA (Fig. 1). It is not immediately clear why insulin synthesis should be preferentially inhibited in islets following both EMC and CB4 infection. However, the inhibition of host cell proteins seen with CB4 might be through the cleavage of the p220 component of the initiation factor eIF-4, as is the case with poliovirus [75]. At a later stage, if cytolysis takes place there will be a general and unregulated loss of islet cell protein synthesis.

Picornaviruses and interferons

As is well known the diabetogenic variants of EMC virus replicate to a high titre in the pancreas of mice and induce low levels of interferons, in contrast to the non-diabetogenic strains which replicate poorly and are high interferon inducers [30]. The interferon system seems therefore to be critically important in the development of diabetes following EMC infection.

It has also been shown that natural isolates of CB4 contain both interferon sensitive and non-sensitive variants of virus which modulate virulence [76]. However, it is much less certain whether or not interferons affect the response of beta cells to CB4 infection. The situation in islets is particularly complicated since interferons α and β may impair insulin biosynthesis directly in vitro [77], and reduce (pro)insulin mRNA (N. D. Portwood, personal communication). These effects could be in response to an interferon-induced activation of endoribonuclease which would depress mRNA in islets. Alternatively interferons could induce an increased phosphorylation of the initiation factor eIF-2, which would depress protein synthesis. Although EMC virus undoubtedly increases the production of interferons in islets, there is no evidence for similar increases in interferons in islets infected with CB4, as measured by immunoassay techniques (K. W. Taylor, personal communication). Moreover, CB4 variants, which attack islets do not affect the levels of the mRNA for the enzyme 2,5-A synthetase which is an indirect mediator of interferon activity. It appears therefore that interferons are much less important in altering islet function after CB4 infection than is the case with EMC virus.

Induction of other proteins in islets by viruses

Cytokines such as interferon, interleukins (IL) and tumour necrosis factor are released in increased amounts following virus infection and they alter the immune response by regulating the expression of beta-cell antigens and thus contribute to tissue inflammation.

In an earlier report reovirus was shown to cause a direct upregulation of HLA class I molecules in islets in vitro [78]. In human insulinoma cell lines measles and mumps virus induced the production of IL-1 and IL-6, and an increased expression of class I and class II antigens [79]. Moreover, a recent study showed that mumps, rubella and CB4 viruses enhanced class I expression in human fetal islets [80]. This is an important area in which further studies are needed.

Autoimmunity and viruses

Diabetes is considered by many to be a chronic autoimmune disease with exposure to an environmental factor being necessary to provoke the immunological reactions which lead to islet cell destruction. It is of importance to investigate which virus or viruses might provoke the immunological response leading to beta-cell destruction. Several antibodies have been associated with diabetes, many of them detectable some years before the onset of the disease e. g. ICA, islet cell surface antibodies (ICSA), insulin antibodies, and antibodies to the 64 kDa antigen. In addition to the involvement of ICA in autoimmunity, there are known to be direct effects of ICA on beta cells; for example, ICSA are toxic to beta cells in the presence of complement [81] and ICA have been shown to alter insulin synthesis and release from islets in vitro [82, 83].

In man the clearest association between a viral infection and diabetes is that of diabetes in CRS and in congenital CMV infection. CRS is typical of an autoimmune disease by being associated with HLA-DR3 [84] and ICA [85] and occurring in 100% of genetically-susceptible individuals [86]. In CRS 12–20% of affected individuals develop diabetes 5–20 years post-infection [18]. In CMV infection ICA may be present which react with the 38 kDa antigen in the pancreas [21]. ICA have also been detected in association with CB and echo 4 virus infection in man [12, 87]. One report suggested that ICA occur with a higher frequency in those diabetic patients with moderate to high CB4 antibody titres [88]. With mumps infection the ICA induced are not always associated with diabetes [89].

Infection with a number of viruses, including picornaviruses, is associated with insulitis, one of the principle manifestations of diabetes. Monocytic infiltration is indicative of the ongoing autoimmune destruction of beta cells. This has been observed in a number of the case histories listed in Table 2 and in animal models of CB-induced diabetes. Similarly in studies of the pancreas of children dying from fulminant viral infections, insulitis was present in 4 of 5 of the cases attributable to CB viruses in one series [90] and in 4 of 7 cases in another [91].

In animal models, ICSA have been reported in EMC-infected mice prior to diabetes [92] and in mice infected with a variant of CB4 which causes metabolic changes in islets [83]. In addition antibodies against the 64 kDa autoantigen, which is released with inflammatory damage, have also been detected in mice infected with a pancreatropic strain of CB4 [93]. The fact that ICA are not always detected when sought for, reflects perhaps the differences in the capacity of virus variants to induce antibodies.

The mechanisms by which a virus might initiate autoimmunity against beta cells is now the subject of considerable speculation. One possible mechanism by which viruses, and in particular the CB viruses, produce diabetes may be by molecular mimicry [14, 15]. In molecular mimicry, sequence and epitope homologies between viral antigens and host determinants result in the generation of host-specific immune responses. A possible role for molecular mimicry in CB-induced diabetes is sug-

gested from studies of the 64 kDa autoantigen released with inflammatory damage to the beta cells. This autoantigen has been identified as glutamic acid decarboxylase (GAD), the biosynthesizing enzyme of GABA, high levels of which are expressed in beta cells [96]. Kaufman et al. [97] and Bu et al. [98] noted that there was an extensive similarity between the GAD_{65} proteins and the P2C protein of CB4 virus. Human GAD_{65} contains a 24 amino acid residue which shares ten identities and nine similarities with the P2C protein of CB viruses. Both these studies suggest that molecular mimicry may be involved in viral induction of insulin-dependent diabetes. Furthermore, a recent study has shown that GAD_{65} and Coxsackie B4 share 17 amino acid similarities and that there are two regions of sequence homology between GAD_{65} and human heat shock protein 65. An increased antibody reactivity to a CB4 nucleopeptide sharing with GAD_{65} an identical sequence of six amino acids has been found in the serum of insulin-dependent diabetic patients [99].

One model of virus infection triggering autoimmune diabetes in transgenic mice has been developed by Oldstone et al. [100]. LCMV glycoprotein or nucleoprotein was expressed in beta cells under the control of the rat beta-cell specific insulin II promotor gene. A subsequent challenge with LCMV provoked a lymphocytic infiltration restricted to the islets and later induced diabetes. This was explained by the fact that the beta cells expressing exogenous antigen only became a target for the immune system when viral infection provided signals to activate anti-beta-cell T-lymphocyte cytolysis. It is not known whether or not this takes place with other viruses.

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