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Review

The role of viruses in human diabetes

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Abstract. Viruses have long been considered a major environmental factor in the aetiology of Type I (insulin-dependent) diabetes mellitus and recent work has greatly confirmed and extended this role. In addition to the enteroviruses, there are several other viruses which, from time to time, have been considered potential causal agents for human diabetes. With the exception of rubella, their role is not clear.

The relation of enteroviruses with Type I diabetes has only been properly clarified by the use of new technologies, especially those based on polymerase chain reaction methods to identify them in blood.

It is now evident from studies in several countries that enterovirus infection accompanies or precedes the onset of diabetes in many children. It is less certain whether this is true for older persons or for other types of diabetes. Enterovirus infection in pregnancy has also been suggested to cause diabetes in children.

The infection with enteroviruses seems to be linked to the induction of islet-cell autoantibodies as well as to the expression of interferon-α. Both of these events are connected with islet-cell destruction.

It has become increasingly important to establish the nature of the infecting virus in the early stages of diabetes. It seems likely that a number of viruses of the coxsackie or echovirus type are involved, although the nature of the nucleotide sequences responsible for diabetogenicity remains elusive. [Diabetologia (2002) 45:1353–1361]

Keywords Viruses, enteroviruses, Type I diabetes, autoantibodies.

Several important new developments have been made linking the onset of Type I (insulin-dependent) diabetes mellitus with virus infections since the subject was last reviewed over 8 years ago [1]. This review considers these new findings in some detail. The role of viruses in inducing diabetes in animals has been reviewed recently in this journal [2]. It is also not our intention to review in detail the molecular basis for auto-immunity, which has also been dealt with elsewhere and which is believed in most cases to be an integral part of the evolution of the disease [3, 4]. A genetic basis for the susceptibility to Type I diabetes, based on *MHC* alleles is assumed [5].

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Abbreviations: PCR, Polymerase chain reaction; EIA, enzyme immunoassay; GADA, glutamic acid decarboxylase antibodies; I-2A, protein tyrosine phosphatase antibodies; ICA, islet cell antibodies; NTR, non-translated region; CMW, cytomegalovirus.

The viruses involved in human diabetes

As is generally realised, there has been speculation on the possible role of viruses as a major environmental factor in inducing diabetes for well over a century. Long before mumps was recognised as a viral disease, it was suggested that it might be associated with the onset of diabetes [6]. Although mumps is now thought unlikely to be a major factor in the induction of human diabetes, it might have contributed occasionally

Table 1. Viruses associated with human diabetes

Viruses for which there is extensive evidence for association with diabetes	
Enteroviruses	Coxsackie A strains (epidemiological investigations)
	Coxsackie B strains – All six types, especially B4 (extensive epidemiology) associated with autoimmunity. Virus isolated induces diabetes in mice
	Echoviruses (epidemiological investigations). Several types could be involved
Rubella	Follows intrauterine infection in offspring. Diabetes might appear after long time intervals. Islet cell antibodies induce. Vaccination has attenuated risk
Viruses less commonly associated with diabetes	
Cytomegalovirus	Might have a small role in pathogenesis. Cross-reactive epitope with GAD 65
Epstein-Barr virus	Virus associated with autoimmune disease, occasionally with diabetes
Mumps	Many earlier reports but role not confirmed. Vaccination has attenuated risk
Retrovirus	Evidence controversial for humans
Rotavirus	Report of islet autoimmunity in children after rotavirus infection

to its development [7]; i.e. before the widespread use of vaccines against it in children.

There are many other viruses in addition to mumps which are associated with the onset of clinical diabetes. Those of principal interest are listed in Table 1, several of which are dubious, but are listed for completeness. In some instances, the viruses listed could have represented an infection which took place concomitantly with the start of diabetes, and might not have any real aetiological significance. Older studies on these viruses have been the subject of another review [8]. In contrast, most attention has been directed to the picornaviruses (which include coxsackie and echoviruses), where the evidence for the close involvement of these viruses in the diabetic process has become increasingly compelling.

Rubella. There is substantial evidence to implicate rubella as a causative factor in human diabetes. Attention was first drawn to the association between the diseases in a 25-year follow-up study of subjects with congenital rubella in Australia [9]. This was later confirmed in later studies in Australia and in the United States [10]. Accordingly, it seems likely that congenital rubella can induce Type I diabetes. As with mumps, the use of vaccines makes it an unlikely candidate in the aetiology of diabetes in the future.

Rotavirus. There has been a recent report suggesting that rotavirus might induce diabetes in children genetically at risk for diabetes [11]. The children were tested at 6-month intervals after birth. It had been suggested earlier that some strains of rotavirus contain peptide sequences highly similar to T-cells epitopes in GAD and tyrosine phosphatase, so that there was a potential for molecular mimicry. The series was a small one and included only five children who progressed to diabetes.

In a more recent prospective study, no association was found between rotavirus infection and the development of beta-cell autoimmunity [12].

Cytomegalovirus. There has been controversy about the role of the cytomegalovirus (CMV) as a potential causal factor for human diabetes. Although pathological damage to islets with the presence of inclusion bodies has been reported in patients dying with a CMV infection, these patients were not known to be diabetic. Moreover, serological evidence for the presence of CMV in newly diagnosed diabetic patients has been reported by some and denied by others. A detailed prospective study of the non-diabetic sibs of diabetic children, followed until some became positive for islet-cell antibodies (ICA) showed no evidence of CMV virus infections preceding diabetes [13]. Recently, an epitope has been discovered which is shared between CMV and GAD 65 which seems able to induce T-cell cross-reactivity [14] but its real clinical significance has yet to be shown.

Retrovirus. Animal studies using NOD mice have suggested that retroviruses could be involved in the induction of human diabetes. A report has shown that newly diagnosed diabetic patients and their first degree relatives positive for insulin autoantibodies have antibodies which can recognise the retroviral antigen p73 [15]. In contrast the IDDMK (1, 2) 22 retrovirus was not detectable in either mRNA or genomic DNA from patients with Type I diabetes [16].

Epstein-Barr virus. Since this virus has been associated with auto-immune diseases, it has been suggested that it might be associated with Type I diabetes. The suggestion was based on sequence analogies between the *HLA-DQ8b* chain and an epitope in the Epstein-Barr virus, which could lead to molecular mimicry [17]. Although occasional cases of diabetes have been associated with this epitope, it is not thought to be a likely candidate virus for diabetes. Antibody levels against the capsid protein of this virus have been shown to be lower in diabetic patients than in control subjects [18].

Human enteroviruses and diabetes

Human enteroviruses are small non-enveloped RNA viruses which are members of the picornavirus family. They consist of over 60 different serotypes and include the polioviruses, coxsackie A and B viruses, echoviruses and a few numbered serotypes. Infection with different serotypes is common, starting from the first few months of life. The virus frequently causes a viraemia and spreads to many organs, which can include the pancreas. Most of these infections are mild and subclinical.

It is only very recently that a clearer role for these viruses is in the aetiology of diabetes has begun to emerge despite a large volume of work devoted to them. The delay relates to two main factors. Firstly, the time scale for the development of Type I diabetes has until recently, not been properly understood. It was not sufficiently considered that Type I diabetes rarely arises as a sudden phenomenon, and that the pathology leading to it can take many years for full clinical expression [19], including autoimmune events. The long period of latency for the disease to develop meant that studies might need to be pursued over several years long after any initial virus infection was detectable.

Secondly, the identification of enteroviruses in current and previous infections in diabetic patients presented unexpected technical difficulties which have only recently been overcome.

Coxsackie B Viruses. Reports of the diabetogenic effects of other picornaviruses such as EMC virus and foot and mouth disease virus, led to the investigation of whether coxsackie viruses might be more generally involved in the aetiology of human Type I diabetes. The results of an analysis of the sera (tested by a neutralising antibody technique) from 123 Type I newly diagnosed diabetic patients suggested that more of them were positive for coxsackie viruses, particularly for coxsackie B4, than were the control subjects [20].

This method is very sensitive and specific for the serological diagnosis of enterovirus infections. A further study using another 162 patients showed essentially similar results, particularly in the 10- to 18-year age group, whereby coxsackie B4 was the predominant virus present at onset [21].

A large number of other groups have attempted similar studies using populations from disparate geographical areas and a variety of techniques to detect virus antibodies. A majority of these (16 out of 23), have shown that the sera of newly diagnosed patients were more strongly positive for coxsackie B antibodies than were with normal control sera. A minority of workers could detect no differences between diabetic patients and control subjects.

There are many reasons for these discrepancies: the basis of patient selection differed widely; very different methods were used to test for viral antibodies. Most importantly the selection of control subjects had not been standardised. Thus, in some surveys, control subjects from very different geographical areas from those of patients were studied. In addition, the HLA type could have differed between the diabetic and control subjects, which might affect antibody levels and therefore comparisons between the groups. It has also not been sufficiently considered that background infection with enteroviruses in the general population can be high at certain seasons of the year, particularly in temperate countries.

A further problem arises from the existence of enterovirus strains which seem to be specifically diabetogenic, due to nucleotide variations. Antibody based tests might not distinguish between diabetogenic and non-diabetogenic strains, further complicating the interpretation of data.

General problems relating to the identification of viruses by classic antibody based analyses have been discussed in detail [22]. Attention has recently been drawn in particular to difficulties with IgM analyses when diabetic sera were examined [23].

Nevertheless, even allowing for serious methodological limitations, it was evident from epidemiological surveys that enterovirus infection was frequently associated with the onset of Type I diabetes in many instances.

Other enteroviruses studied in epidemiological surveys. Some earlier reports have suggested that both coxsackie A viruses [24] as well as echoviruses could be associated with the onset of Type I diabetes. A further recent report from Cuba has shown that newly diagnosed patients with Type I diabetes were much more strongly positive for neutralising antibodies to echovirus 4 than the control subjects [25].

Seasonal incidence of Type I diabetes. Seasonality in the diagnosis of Type I diabetes was recorded many years ago, with peaks in the autumn and winter months [26]. Many other studies in both the northern and southern hemispheres have confirmed these findings, which have generally been interpreted as coinciding with the time of enterovirus infections. A recent study has also recorded that the time when diabetes associated antibodies first appear also reflects a very similar seasonality [27].

PCR analyses of blood from diabetic patients. The use of polymerase chain reaction methodology enabled viruses to be identified by molecular methods, thus circumventing the indirect approach through antibody based analyses. An additional advantage was that these methods could be extended to delineate virus nucleotide sequences. An analysis of the serum from a small group of very young children at the onset of their diabetes using these methods showed that most (64%) were positive for enteroviruses as opposed to

Table 2. Identification of enteroviruses in blood of diabetic patients by PCR methods

Reference	Year of study	Source of sample	Type of patient investigated	Virus type
[28]	1995	serum	very young children	coxsackie B3B4
[30]	1997	whole blood	adults	coxsackie B3B4
[29]	1999	serum	children younger than 15 years of age	coxsackie B viruses and echoviruses
[23]	2000	whole blood	children and adults	coxsackie B2B3B4
[34]	2000	serum	prediabetic children	not determined
[35]	2000	serum	prediabetic children	not determined
[33]	2000	serum	diabetic children	–
[31]	2001	mononuclear cells	diabetic children	coxsackie B4B5 cells

4% of the control group. The viruses were of the B3/B4 type. Further sequence work using the NTR of the virus, suggested homologies on a limited scale among the diabetic patients. There was a minor degree of clustering in some geographical regions [28].

In a later study from the same laboratory broadly similar results were obtained with a much larger patient group of 110 children younger than 15 years of age and 180 control children matched for age and geographical area [29]. Positivity was especially noted in younger children at the onset of their diabetes. Over the entire age range 27% of the children were positive for enteroviruses compared with 4.9% of the control children. The figure for positivity rose to 37% in children of 3 years of age or younger. These results have been confirmed in a number of other studies in France [30], Sweden [31, 32] and in Australia [33]. In the results from Sweden and France, whole blood was the starting point for the work and the percentage positivity rose considerably (Table 2).

Of note, some adults seemed to be positive for enteroviruses by PCR methods at the start of their diabetes and there was limited evidence for virus persistence in the studies from France.

Others have detected enteroviruses by PCR methods in the serum of children genetically at risk for diabetes some months or years before the clinical condition developed [34, 35].

Significance of tests to detect viruses. It is important to realise that PCR methodology and traditional antibody techniques for virus detection are measuring very different aspects of a viral infection. PCR applied to blood or serum essentially indicates a viraemia, usually lasting a few days. In contrast, tests for virus antibodies can only indicate a previous virus infection. Depending on the test, the infection could have been recent or at a relatively distant time in the past. As with tests for virus antibodies, there are also a number of potential problems in using the PCR tests for detecting viruses.

Contamination can create difficulties as well as cause false positives [36] and repeated freezing and thawing of specimens can also interfere with results [34]. Nevertheless, results using these tests from three western European countries show consistency, especially in diabetic children compared with control subjects with very low levels of infection. They reinforce the conclusions of the earlier antibody studies suggesting that enterovirus infection is associated with the development of Type I diabetes.

Extraction of serum. It has been suggested that cellular elements in blood could sequester enteroviruses [37]. If so, then the use of whole blood or of blood extracts might better reveal viraemia. The percentage positivity of patients tested for enteroviruses at the onset of diabetes is greatly increased in the Swedish study, where monocytes were used, as well as in the French study which used whole blood. In the Swedish study, as many as 71% of children tested were enterovirus positive.

Age range of patients studied. Although Type I diabetes has usually been considered a disease affecting juveniles, it can present at any age. Studies to determine whether virus infection accompanies the onset of diabetes, however, have generally been confined to subjects younger than 15 years. In the previous studies carried out in the United Kingdom [20], most patients with diabetes of sudden onset and presenting with ketosis were older than 20 years of age (80%). Although this might have suggested that older age groups showed virus positivity at onset, this problem does not seem to have been systematically studied until very recently.

In one set of results using PCR methodology, it has been shown that positivity for enteroviruses was quite commonly present in adult insulin-dependent diabetic patients at onset [30]. In a small number of patients, previously diagnosed with insulin dependent diabetes, enterovirus sequences could also be detected, raising the possibility of a continuing infection. Non-insulin dependent diabetic patients were negative for enteroviruses when tested in this system.

Enterovirus infection in individual case studies. A number of cases have been reported in which proven

enterovirus infection has been associated with the onset of insulin requiring diabetes. In an earlier review of 11 such cases who were mainly children, all six coxsackie viruses were implicated. In two instances, viruses were isolated from the patients and shown to be diabetogenic in mice [38, 39]. More recently, an infant acquired diabetes at the age of 14 months after clinically diagnosed enterovirus infective episodes [40].

Enterovirus infection and diabetes among sibs of families. There are instances where the diagnosis of diabetes in one member of a family has been rapidly followed by its appearance in other members of the same family [41, 42]. The viruses involved were of coxsackie B type. Using PCR methodology, two 14 month old identical twins infected with echovirus 6 variants acquired diabetes within 12 days of one another [43].

Such cases indicate that infection is likely to have been from a common source within the family. They add further weight to the strong epidemiological evidence for the association of enterovirus infection with the onset of Type I diabetes.

Nucleotide sequences of enteroviruses identified in blood. Nucleotide sequences have been studied by several groups in enteroviruses identified in the blood of diabetic patients. Using the non-translated segment of viruses in diabetic children a study showed that there were common nucleotide sequence in some groups of patients. Thus at position 229, adenine was present instead of the guanine of the prototype coxsackie B3 or the cytosine of coxsackie B4 in five out of six patients [28]. Although other changes were recorded, the sequences were closely related to coxsackie B3 and B4 in general.

In a later study from the same group, 110 children with diabetes were studied at the onset of their disease for enterovirus sequences and the non-translated portion of the genome were further analysed after amplification which suggested that viruses found in the diabetic children were distributed throughout the echo and coxsackie groups based on affinities between the structure of groups [29]. Studies from a small group in France gave similar results [30]. Again, changes at position 229 were noted in two patients. Generally, however, changes in nucleotides did not coincide with those detected in the British series. In a later study from this group using PCR techniques they confirmed that coxsackie B2, B3 and B4 were the chief infecting viruses in new and established diabetic patients. A similar result was obtained in a study of newly diagnosed Swedish children [31] whereby the infecting viruses showed homologies with coxsackie B4 and B5. However the exact serotype cannot be determined from studies on the non-translated portion of the genome because analysis of virus capsid proteins might be needed.

Based on the limited evidence available, a wide range of enteroviruses of the echo or coxsackie type are clearly associated with Type I diabetes, with perhaps coxsackie B4 especially common. It is not possible, with the work from serum, to identify those nucleotide determinants which confer diabetogenicity on the virus. The experimental problems are compounded by the known high rate of natural mutagenesis amount these viruses.

Nucleotide sequences of viruses causing experimental diabetes or changes in islets. Several groups have done studies to determine the complete nucleotide sequences of viruses which attack islets under experimental conditions. In the first of these studies [44] a coxsackie B4 variant which caused metabolic changes in islets, including human islets, was studied and its structure compared with the prototype CB4 virus. This variant had been passaged in pancreatic tissue and was mildly diabetic in mice. A total of 25 nucleotide differences were noted in the diabetogenic virus compared with the prototype. Six of these occurred in the non-translated region of the genome and 19 in coding regions producing seven amino acid changes. It was suggested that nucleotide changes in the non-coding region of the genome are of particular importance in altering replication rates.

In a later report [45] rather more extensive changes in the genome were reported in a more markedly diabetic strain of the coxsackie B4 virus, which has been derived from a human source (E2 strain). In this strain there were 111 amino acid substitutions, most of which were located in the non-capsid region. Capsid proteins, VP1 and VP2 showed the largest changes from the prototype. The very large number of changes made it difficult to define which nucleotides conferred diabetogenicity.

Another report examined another coxsackie B4 variant which directly affects human pancreatic islets in tissue culture. This strain can produce a persisting infection of islets with concomitant effects on insulin secretion and proinsulin synthesis [46].

From these studies, it is clear that much further work needs to be carried out to decide those nucleotide structures which cause enteroviruses to be diabetogenic. It seems likely that different sequences of nucleotides might be involved for example in receptor binding and in replication phenomena. More work using site directed mutagenesis and recombinant viruses will be needed to establish the responsible genomic structures.

Interferons and T-cell responses to enteroviruses. Type 1 interferons, including interferons-α and -β, are the most potent antiviral cytokines. They are part of the innate immune system which is activated early during an infection before adaptive immune responses are induced. A type 1 interferon response is evoked by

various viruses in difference cell types and their presence suggests an ongoing virus infection. Interest was particularly focussed on type 1 interferons when it was found that beta cells, but not other islet cells, express interferon-α in diabetic patients at autopsy [47].

It was later shown that interferon-α was raised when the serum of adult diabetic patients was tested [48]. In more recent studies, increased concentrations of interferon-α were again detected in the blood of a wide group of new and treated Type I diabetic patients, as was interferon mRNA in blood cells. The increased interferon concentrations seemed to be correlated with enterovirus infection [23]. It was thought that interferon production was a reliable indicator of ongoing virus infection, mainly from coxsackie viruses. Coxsackie B4 seems able to induce the synthesis of interferon-α in human beta cells in vitro, thus mimicking the expression of this cytokine in the pancreas of Type I diabetic patients [49].

An increased expression of the interferon-alpha-inducible effector molecule (2'5' oligoadenylate synthetase) has also been described in diabetic patients [50]. These studies emphasize the potential importance of interferon-α as a factor linking virus infection with islet autoimmunity. Increased T-cell proliferation to enterovirus antigens has also been reported in Type I diabetic patients [51, 52, 53] which could reflect increased exposure to enteroviruses in these patients. Cellular immune responses are considered to be an obligatory part of the beta-cell damaging process. One possibility is immunological cross-reactivity between viral and beta-cell proteins, which has been investigated [54].

Enterovirus-induced T-cell responses also generate several other proinflammatory cytokines (e.g. interferon-α), which can promote the activation of autoreactive T-cell clones [55]. Very recently an enhanced production of interferon-γ was noted in T-cells from newly diagnosed Type I diabetic patients using coxsackievirus B4 antigens [66].

Further recent epidemiological studies

Prospective studies. Most studies which have sought to evaluate a role for viruses in the aetiology of human diabetes have been retrospective. Prospective studies have the advantage that they can include the earliest initiating stage of the process.

The first prospective studies were carried out under the Childhood Diabetes in Finland (DiMe) scheme. They were based on a series of non-diabetic children who were the siblings of Type I diabetic patients. These children were followed for several months until they became positive for autoantibodies associated with diabetes or until they became clinically diabetic.

These studies showed that enterovirus infections, diagnosed by antibody assays and by the presence of viral RNA in serum, were more frequent in such siblings that in siblings who remained non-diabetic (or antibody negative).

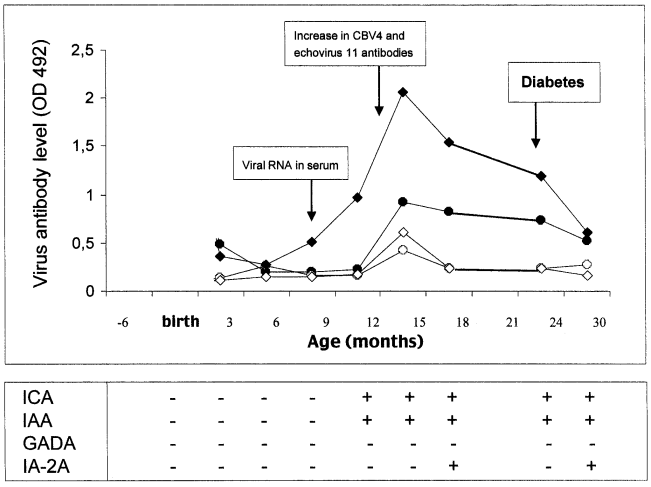

Fig. 1. Enterovirus infections and the appearance of autoantibodies and clinical diabetes in a child participating in the Finnish Diabetes Prediction and Prevention (DIPP) trial. Enterovirus antibody levels against different enterovirus antigens as measured by EIA from serial serum samples are shown in the upper panel. Twofold or greater increases between the samples were considered to be significant. The presence or absence of autoantibodies during the follow up is indicated by (+/–) in the lower panel. Level of coxsackie virus B4 IgG shown by ●; B4 IgA ○. Level of echovirus 11 IgG ◆; IgA ◇

Moreover, infections were also clustered immediately preceding the appearance of autoantibodies even several years before the diagnosis of clinical diabetes [56, 57, 58, 34]. The frequency of other virus infections such as cytomegalovirus, adenovirus and Epstein Barr virus did not differ between the groups [13, 56, 59].

These findings have been confirmed in another prospective study, the Finnish Diabetes Prediction and Prevention trial (DIPP) whereby newborn children with diabetes-associated HLA-DQ alleles are followed from birth [60]. Enterovirus infections were more frequent in children who became autoantibody positive than in the control children. Again infections showed clustering at the time when autoantibodies first appeared [35, 61]. In addition, one prospective study from Australia indicated that rotavirus seemed to be associated with diabetes, but the role of enteroviruses was not analysed in detail [11].

Taken together, these studies have confirmed the importance of enterovirus infections as a risk factor for subsequent diabetes and have linked virus infection with the expression of islet-cell antibodies (Fig. 1). The episodes of virus infection in a child participating in the prospective DIPP study are summarised here.

Observations started in the mother with maternal serum taken 6 months before birth and continued until the child progressed to diabetes at the age of 2 years.

Two enterovirus infections were diagnosed using a wide panel of antibody assays (both EIA and neutralisation assays) and viral RNA in serum was detected by RT-PCR. The first infection at the age of 9 months was viraemic (enterovirus RNA in serum). The infection was caused by a coxsackie virus B2 serotype and was identified by using a neutralising antibody assay. The second infection was diagnosed at the age of 15 months by an increase in antibody levels as measured by EIA. Autoantibodies appeared 3 months after the first infection at the age of 12 months and remained positive until diabetes was diagnosed. In this child the appearance of ICA and IAA coincided with the first enterovirus infection by a coxsackie B2 serotype followed by the manifestation of Type I diabetes a year later. From this type of evidence we believe that the likely course of events for the genesis of Type I diabetes in children is that multiple infections with enteroviruses starting early childhood evoke an autoantibody response leading to diabetes.

The first infection could initiate the process which is later accelerated by serial infections in childhood. Such infections might easily be subclinical and with little generalised effects on the health and well being of the child until diabetes supervenes. The results described do not rule out that such infections could also sometimes begin during pregnancy as is discussed below.

Diabetes and enterovirus infections in pregnancy. Some earlier reports have shown an association between congenital rubella and diabetes arising later in life [9]. This work suggested that some other viruses might be similarly involved if infection took place during pregnancy. Using a childhood diabetes register in Sweden, Dahlquist was able to trace sera stored over a 10 year period, which was obtained from the mothers of diabetic children. Both IgM and IgG tests showed a considerable excess of antibodies against enteroviruses in the mothers of the diabetic children as opposed to the sera from the control mothers. In this study the viruses tested for were coxsackie viruses and echoviruses [62].

The results indicated that enterovirus infection during pregnancy might well anticipate diabetes in the child. In a different series, coxsackie B2, B3 and B4 were looked for by using IgM analyses [63]. Mothers of diabetic children, whose blood was sampled at the time of birth, were more frequently positive for coxsackie B3 than the control subjects.

In a Finnish series the levels of antibodies to enteroviruses were greater in the mothers of children who had diabetes before the age of 3 years than in a control group. This was especially so when comparing IgM measurements relating to coxsackie B5 [56]. The serum was taken 3 months into pregnancy as part of a programme screening for infectious diseases. In a later study in Finland using children with *HLA-DQB1* alleles and who were positive for islet-cell antibodies, enterovirus infections in pregnancy did not seem to be more common than for mothers in the control group [35]. Here blood was sampled at the end of the first trimester and samples were used from cord blood.

However, further work carried out on the mothers of Swedish diabetic children again confirmed that there was an excess of mothers having an enterovirus infection 3 months into pregnancy. In this small series 6 out of 85 mothers were positive for enteroviruses as opposed to only one out of 172 control subjects [64].

A report on neonatal diabetes suggested that diabetes could have followed a maternal echovirus 6 infection acquired during pregnancy. In this case islet-cell antibodies were detected in the child [65].

Conclusions

There can now be little doubt that enterovirus infection either accompanies or precedes the onset of Type I diabetes in young people in many instances. Recent data using PCR methodology on blood samples suggest that perhaps a majority of children with diabetes can present in this way. There are also indications that Type I diabetes first appearing in adults is similarly accompanied by such infections but such work needs to be confirmed. There have been several suggestions that enterovirus infection during pregnancy might initiate the events leading to childhood diabetes; however, not all investigators have yet confirmed these findings. Perhaps the high rate of infection with enteroviruses among newly diagnosed diabetic patients merely reflects an increased susceptibility of such patients to infective processes. There are powerful reasons for rejecting this argument. The tendency to virus infection among diabetic patients is not shown for other viruses. Neither is infection related to metabolic decompensation, nor to high blood glucose concentrations; it is not seen in Type II diabetic patients. Moreover, only certain strains of enterovirus, derived from human diabetic patients can evoke diabetes in animals. This specificity must relate to changes in nucleotide sequences which have yet to be determined. The limited information available in humans suggests the viruses are of the coxsackie or echovirus type.

In summary, we believe that evidence now strongly suggests that enterovirus infections can serve as a major trigger for Type I diabetes in the young, by processes which are at present uncertain, although these must clearly involve the induction of islet-cell antibodies. This evidence involves seasonal incidence studies, numerous epidemiological surveys, (especially those using PCR methods), and detailed descriptions of individual clinical cases of enteroviruus infection accompanying the onset of diabetes. Crucially, the induction of islet cell antibodies seems to be relat-

ed in time to episodes of enterovirus infection. This can take place long before diabetes is clinically evident. It is seen when patients and control subjects are carefully matched for *HLA*-type. Although not discussed in this review, there are a considerable number of published animal studies on the effects of these viruses which support our contention. We cannot exclude the involvement of some other viruses in the diabetic process, as for example rubella, but we think that this is an uncommon event. If further investigations continue to support the hypothesis that enteroviruses induce diabetes, then intervention therapy, for example by vaccination or by drug treatment, will become an urgent necessity.

Sources. This review is based on the relevant literature published in the English language during the period 1990 to June 2002 and on important earlier contributions.

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