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Is Cytomegalovirus Associated With Type I Diabetes? A Meta-analysis and Evaluation of Plausible Biological Models

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Is Cytomegalovirus Associated With Type 1 Diabetes? A Meta-analysis and Evaluation of Plausible Biologic Models

by

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ACKNOWLEDGEMENTS: .................................................................................................................. ii

JOINT INTRODUCTION: .................................................................................................................. 1

PAPER 1 - BIOLOGIC THEORY ..................................................................................................... 3

INTRODUCTION .............................................................................................................................. 3
  Type 1 diabetes is an autoimmune disease ................................................................................. 4
  Tolerance and its breaking ........................................................................................................... 5

TYPE 1 DIABETES ........................................................................................................................... 7
  Genetics ........................................................................................................................................ 9
  Pathogenesis ............................................................................................................................... 9
  Animal models ............................................................................................................................ 11

POSSIBLE CAUSES OF TYPE 1 DIABETES .............................................................................. 12
  Coxsackie B virus ....................................................................................................................... 12
  Congenital rubella and others ..................................................................................................... 13
  Cytomegalovirus - general ......................................................................................................... 14

EVIDENCE FOR ASSOCIATION BETWEEN CMV AND TYPE 1 DIABETES ............................. 17
  Autoimmune mechanisms ......................................................................................................... 18
    Molecular mimicry .................................................................................................................. 18
    Release of self-peptides ........................................................................................................... 20
    Polyclonal activation ............................................................................................................... 21
    Inhibition of protective lymphocytes ...................................................................................... 22
  Non-autoimmune mechanisms ............................................................................................... 24

CONCLUSION .................................................................................................................................. 25

REFERENCES ................................................................................................................................. 27

META-ANALYSIS ............................................................................................................................. 32

BACKGROUND AND SIGNIFICANCE: ......................................................................................... 32

METHODS ....................................................................................................................................... 35
  Study selection ............................................................................................................................ 35
  Data abstraction ......................................................................................................................... 35
  Statistical analysis ..................................................................................................................... 36

RESULTS .......................................................................................................................................... 38

PRINCIPAL ANALYSES AND SUBANALYSES ............................................................................ 39
  Overall ....................................................................................................................................... 39
  Stratified analyses: ..................................................................................................................... 40
    IgG ........................................................................................................................................... 40
    IgM ........................................................................................................................................... 40
    PCR ........................................................................................................................................... 41
    Congenital CMV ....................................................................................................................... 41
    Overall with ICA ....................................................................................................................... 41
  Sensitivity analyses ..................................................................................................................... 42

Publication bias ............................................................................................................................. 42

DISCUSSION: .................................................................................................................................. 43
  Directions for further research: ..................................................................................................... 48

TABLES AND FIGURES ................................................................................................................ 50

REFERENCES: .............................................................................................................................. 54

CONCLUSION: ............................................................................................................................... 58

REFERENCES FOR INTRODUCTION AND CONCLUSION .......................................................... 59
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Joint Introduction:

As medicine has moved toward emphasizing evidence-based medicine in the making of practice guidelines, methods for synthesizing data in the literature have become more important. Meta-analysis is one technique that has gained popularity in recent years. Meta-analysis allows a quantitative analysis of the body of literature on a subject, with the goal of calculating a summary estimate of effect and/or assessing sources of heterogeneity between studies already done. Meta-analysis provides a systematic method of reviewing a literature in an unbiased fashion.

The use of meta-analysis has increased so much in the past ten years that a search of MEDLINE with the restriction publication type: meta-analysis yields 774 citations for the year of 1999, compared with 279 meta-analyses of randomized trials published in the entire decade of the 1980s.[1] With this increase in use, some have voiced concern, arguing that meta-analysis leads to a false sense of conclusiveness about a literature. In response, emphasis has been placed on statistical methods, and the reporting of heterogeneity, but the controversy continues. Other concerns, such as publication bias, are very real and need to be addressed by every meta-analyst.

While the use of meta-analysis for summarizing randomized studies is now well established, the use of meta-analysis to synthesize observational studies remains more controversial. Recent work has allayed some of the fears of bias and systematic overestimating that critics had initially raised. In 2000, Concato concluded that; "The results of well-designed observational studies (with either a cohort or a case-control design) do not systematically overestimate the magnitude of the effects of treatment as compared with those in randomized, controlled trials on the same topic." [2] Concato
considered meta-analyses and the results of large randomized controlled studies in five health areas, and found that the results were remarkably similar between these two study types. Noting the necessity of a rational approach to synthesizing observational studies, the CDC sponsored a committee, the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group, which published in 2000 a checklist for meta-analyses of observational studies.[3] This is important because some questions about human disease can only be studied by observational studies; in some cases a randomized study would be unethical, in others it would be impossible for practical reasons. The etiology of type 1 diabetes is one such area where observational studies are required to study the disease in humans.

This thesis uses meta-analysis to evaluate the possible association between cytomegalovirus and type 1 diabetes. The first paper is a discussion of the theoretical aspects concerning the biologic plausibility of such an association, taking into account animal models of the disease. The second paper uses meta-analysis to review the literature that has accumulated about this question. The review of epidemiological evidence can augment discussion of the biology of diabetes, while biologic theory is needed to interpret the results of the epidemiological work.
Paper 1 - Biologic Theory

*Introduction*

[They] Make war upon themselves; blood against blood,
Self against self: O, preposterous And frantic outrage,

KING RICHARD III ACT: II SCENE: IV[1]

Like standing armies everywhere, the "armies" of the body that guard against invasion always have the potential for self-attack. Called "horror autotoxosis" by an early researcher, the "frantic outrage" of the body making war on itself is what we now call autoimmune disease.[2] Researchers have long wondered about the mechanisms that keep the immune system, the body's standing army, from turning its force against itself, and what sometimes causes breakdown of this system. In the past fifty years, many diseases of previously unknown etiology have been classified as autoimmune diseases, including type 1 diabetes.

There is still much that we do not understand about autoimmune disease. General questions, like why the prevalence of autoimmune syndromes has increased in recent years in the Northeastern US and Europe still do not have answers.[3,4] The explanations are puzzling and contradictory: some point to decreased childhood infections, while others blame increased infections or environmental toxins. On a disease by disease basis, genetic and environmental risk factors have been identified for some autoimmune diseases, but conclusive etiologies have yet to be found for most. Although type 1 diabetes has received much attention and research dollars, it is still unclear why one person might be struck with diabetes, while her brother remains healthy.

In this paper I will examine the current theories of the pathogenesis of type 1 diabetes, which is characterized by permanent autoimmune destruction of the beta cells.
of the pancreas, and the evidence linking cytomegalovirus (CMV) infection to this disease. Areas for further research will be identified.

**Type 1 diabetes is an autoimmune disease.**

The term diabetes¹ was coined in the second century CE by Aretaeus of Cappadocia, but the syndrome was described three millennia earlier in China. After naming the disease, it took another sixteen centuries before the link between sweet urine and elevated blood sugar was made, and it was during the twentieth century that the discovery of insulin paved the way for effective treatment of type 1 diabetes.[5] In the last half century, it has become recognized that type 1 diabetes mellitus, also called juvenile diabetes and insulin dependent diabetes mellitus (IDDM), is an autoimmune disease that leads to destruction of the beta cells found in pancreatic islets.

Type 1 diabetes is a disease that usually manifests in childhood, and is the most common chronic disease that plagues children and young adults[6]. Its incidence has risen in the past quarter century in the developed world[4], and an estimated 1.3 million Americans suffer from type 1 diabetes.[7] Although the peak incidence is in the first and second decade of life[8], 5-10% of adults over 40 who present with diabetes for the first time have an autoimmune etiology.[9] This disease can be a part of a syndrome of multiple autoimmune diseases, perhaps indicating a common diathesis. Specifically, autoimmune thyroiditis, and Addisons disease (adrenal insufficiency), are associated with type 1 diabetes and shared genetic traits.[10] Although much has been learned about the

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¹ *Diabetes* literally means "to pass through" or "siphon", a reference to the polyuria characteristic of the disease. Diabetes mellitus ("honey") is the type of diabetes discussed here - there are distinct syndromes that do not relate to glucose regulation.
pathology and treatment of these diseases, our knowledge about prevention and causes is woefully lacking.

**Tolerance and its breaking**

In order to understand autoimmune disease, it is important to be familiar with the normal mechanisms of immunity and tolerance. The immune system is remarkable for its specificity and diversity. B and T cells, the 'soldiers' responsible for specific memory, work in concert to neutralize and eliminate foreign antigens from the body. Because T cells are responsible for the pathology of type 1 diabetes, I concentrate here on T cell development, although a very similar process happens for B cells. Through genetic recombination at the somatic level, \(10^{12-14}\) T cells with different specificity are generated during fetal life.[11] Because this system is random, it would be expected that a great number of T cells are produced which are particular for self antigens. Several mechanisms exist to ensure that these cells do not go on to attack these self antigens.

In the thymus of the developing animal all T cells are presented with a large array of self antigens in conjunction with MHC class II molecules. Those whose T cell receptors (TCR) bind to those antigens with a high degree of affinity are signaled to die, and so do not reach circulation. This central mechanism of tolerance, thymic deletion leaves, however, those cells specific for self antigens that were not presented in the thymus, and those whose T cell receptors bound with only moderate affinity. In order to ensure that these cells do not cause destruction, there are peripheral regulatory mechanisms.
In addition to the binding of the TCR, a co-stimulatory signal is required to activate T cells outside of the thymus. In its absence the T cell is deactivated and a state of anergy is induced. There are only a few types of cells which provide the costimulatory signal needed for activation - these cells are referred to as 'professional' antigen presenting cells, and include dendritic cells, macrophages and B cells. In order to produce a T-cell response, invaders, such as viruses, must be presented in the presence of co-stimulation. Self antigens, on the other hand, are usually presented without co-stimulation and so induce peripheral tolerance.[12] Nonetheless, a small number of auto-reactive cells are always present and may even be active.

In recent years the importance of certain cell types in regulating these auto-reactive cells and the normal immune response has been recognized. In the 1970s and early 1980s, the concept of antigen specific regulating T cells, called suppressor T cells, was widely believed. These cells were postulated to secrete soluble factors which blocked antibody/receptor sites, inactivating T and B cells. The idea of a soluble blocking factor was not proven, and this theory was abandoned by most in favor of a theory of cytokine classes.[13] This currently accepted theory divides helper T (CD4+) cells into either Th (T Helper) 1 or 2 subtypes based on their cytokine profile. Th 1 type cytokines, IFN-gamma, TNF beta, IL-2 and IL-12, are associated with a cell mediated immune response, while Th 2 cytokines, such as IL-4, IL-10, are associated with a humoral response. Each cytokine has distinctive functions, and some inhibit the alternative Th sub-type, enforcing the domination of either Th 1 or Th 2 cells in any given immune response.[11, 14, 15] Whether one response or the other is initiated is dependent on the route and nature of antigenic exposure. Janeway writes that: "Large amounts of peptides that achieve a high
density on the surface of antigen-presenting cells tend to stimulate Th1 cell responses, whereas low-density presentation tends to elicit Th2 cell responses.\cite{16} The direction of the immune response has important implications for autoimmune disease. Pathologic insulitis (associated with type 1 diabetes, and discussed below) is characterized by a Th1 profile of cytokines, and Th2 cytokines are protective. Therapies which slow or reverse insulitis in mice are associated with increased Th2 cytokine levels locally.\cite{17}

The theory of the Th1/Th2 dichotomy is generally accepted, but the suppressor T cell hypothesis has also regained support. Evidence points towards antigen specific subsets of T cells that suppress other T cells. Neither how these cells accomplish this, nor the relationship of these cells to Th2 cells is well defined\cite{13, 18, 19}. These theories are not mutually exclusive, and much remains to be worked out regarding these models, but the importance of active methods of immunosuppression is clear. One place where this can be seen is in the seemingly paradoxical relationship between immunodeficiency syndromes and autoimmune diseases. Autoimmune diseases are more common in congenitally immune deficient humans, as well as in those who develop immune deficiency later.\cite{20} This will become important later on when we discuss the pathogenesis of type 1 diabetes.

**Type 1 Diabetes**

Despite the seemingly endless potential self targets, and possibilities for the disruption of self-tolerance, autoimmune diseases are relatively rare. Of the several types of autoimmune disease, type 1 diabetes most neatly fits into the category of T cell mediated organ specific autoimmunity. Other diseases that are believed to have this pathogenesis include multiple sclerosis, experimentally induced allergic
encephalomyelitis, and common rheumatoid arthritis.[16] Helper T cells play a primary role in autoimmune thyroiditis [21] and Addison's disease, but autoantibodies seem to be more important in these diseases than they are in type 1 diabetes. Type 1 diabetes, and other cell mediated diseases are more difficult to study than antibody mediated diseases because T cells cannot be transferred to non-identical animals, and because it is difficult to identify which antigen a T cell clone is specific for. [16]

Type 1 diabetes is not unique among cell mediated autoimmune diseases in having an unclear etiology. MS has a definite geographic variation, which has been linked to an endemic viral risk, but the evidence is thin. An experimental disease in mice which is very similar to MS is caused by an encephalopathic virus, but the search for such a virus in humans is ongoing.[22]

Most autoimmune diseases affect a higher proportion of females of reproductive age than men. MS has a female to male ratio of 10, and rheumatoid arthritis a ratio of 3 [16]. The mechanisms of the sex ratio of autoimmune diseases are unclear.[23] Some cytokines are influenced by sex hormones[24], and estrogen can cause immune suppression[25], but there may be other processes at work. Diabetes differs in that is affects males and females about equally. Diabetes may lack this ratio because of a strong environmental and/or genetic risk associated with it. An example that illustrates this theory is Reiter's syndrome, which affects males more frequently than females and has a strong genetic component and association with certain bacterial infections.[26] One of the major animal models of type 1 diabetes, the NOD mouse discussed below, has a large female to male sex ratio for diabetes.[27-29] In this way the NOD mouse may not be a good model of human diabetes.
Genetics

More than twenty genes have been associated with type 1 diabetes, including insulin and certain human lymphocyte antigen genes. European Americans with type 1 diabetes are more than twice as likely to have the MHC II HLA subtype DR3 or DR4 than the general population.[7] The connection between HLA type and autoimmune disease has been found in other autoimmune diseases, including those mentioned above. The MHC II protein is involved in the presentation of peptides to the immune system. It is possible that the alleles associated with diabetes code for MHC molecules that select diabetogenic proteins to present.[8] Despite the large number of genes associated with diabetes, identical twins of Type I Diabetics have only a 30-70% risk of developing the disease themselves[7, 16, 30-32], indicating that a genetic predisposition may be necessary but not sufficient, and that the disease is multi-factorial.

Although there is large variation in disease rate by ethnic group, with Finnish children at a 60 fold higher risk than Korean children, [33] immigrants approach their host countries risk after a long stay.[34] Environmental factors have long been sought to account for these variations in risk. Viral infections, dietary factors and breastfeeding have all been implicated in the geographic variability of type 1 diabetes. These possibilities are described at more length below.

Pathogenesis

Pathologically, type 1 DM is characterized by destruction of the beta cells of the pancreas which produce insulin, a destruction preceded by a period of "insulitis" (infiltration of lymphocytes in the pancreas) that develops well before clinical symptoms
are present. Although this infiltrate is dominated by B cells, this disease is T cell mediated, as evidenced by the transferability of disease by T cells but not by antibodies. [35]. Experts disagree whether it is CD4+ (T helper) or CD8+ (cytotoxic T) cells that initiate and maintain the destructive process, perhaps because of the difficulty in collecting a purified sample of one type of T cell.[35, 36]

Nor is it entirely clear which antigens are important in the pathogenesis of this disease.[6] Autoantibodies to a number of islet cell components are present in nearly all type 1 diabetics, and are able to predict disease with a high degree of sensitivity and specificity. Cytoplasmic islet cell antibodies (ICA) have been used as predictors of disease in first degree relatives of those suffering from type 1 DM, but the assay is expensive and difficult to standardize.[32] More recently, insulin autoantibodies (IAA), glucamate decarboxylase autoantibodies (GAA) and ICA512bdc antibodies have been shown to be highly effective in predicting life time diabetes risk in first degree relatives. Verge and others found that: "the five year risk was 0% if no auto-antibody was positive, 15% if only one was positive, 44% if two were positive and 100% if all three were positive".[32] Despite the specificity of these antibodies in predicting disease, it is unlikely that the disease is caused by antibodies. Antibodies do not induce disease when transferred from a diabetic animal to a healthy one. Auto-antibodies may be no more than markers of the destructive process.[8] Determining what antigens the pathologic T cells are specific for has proved difficult. One reason is that as the inflammatory process progresses, it is likely that there is antigenic spread.[37] As Cohen puts it, when analysis is done in diabetic animals or humans; "The autoimmune process by then is winding down and it is difficult to sort out primary and secondary processes." [38]
The theoretical model of pathogenesis currently favored includes several environmental insults that either initiate or accelerate pancreatic damage or precipitate clinical symptoms. It is not until 80% of islet cells have been destroyed that diabetes develops, long after the original insult occurred that initiated autoimmunity. As will be discussed later, CMV is a suggested cause of early insult that predisposes to further destruction [39] or accelerates preexisting autoimmunity.[40]

**Animal models**

Several rodent strains that develop diabetes in ways similar to humans have been invaluable in the exploration of the pathogenesis of diabetes. Rodents are good models for CMV because they contract an analogous virus to human CMV, called Murine CMV, which is very similar to HCMV in viral structure and clinical effects. The Non-Obese Diabetic (NOD) mouse was developed in the late 1970's by Makino and his colleges in Japan [41], and has subsequently been used throughout the world to model human type 1 diabetes. This strain develops diabetes spontaneously, and has a polygenic inheritance pattern of susceptibility, which matches the human disease.[42] Like human type 1 diabetes, these genes include MHC class II (HLA) genes and non MHC genes including those that code for islet cell proteins.[28] Other similarities include the transferability of disease by hematopoetic stem cells, a similar pathologic pattern of insulitis, the presence of a variety of anti-islet cell antibodies, and dependence on T cell mediated destruction.[28] In these mice, insulitis develops at 4-5 weeks in 100% of females, and at 30 weeks in 90% of males.[42] This infiltrate has been called "non-destructive peri-insulitis", and by 6 months 80-90% of female mice, and only 10-40% of male mice, have
destruction of islet cells, and clinical symptoms of diabetes.[28] Clearly, there is a
difference between the "non-destructive" insulitis that all of the NOD mice share, and the
pathology that leads to the clinical syndrome of diabetes and beta cell destruction in
some.[30]

The Bio Breeding rat also develops diabetes spontaneously, but it differs from the
NOD mouse in that it has severe T cell lymphopenia, which may be the result of thymic
dysfunction.[9] Other mice, including the BALB/C mouse, have been used for diabetic
research, but do not develop diabetes spontaneously.[27] It is quite possible that the
relationship between diabetes and a given infection could be modified by the genetic
background of the research animal.

Possible causes of type 1 diabetes

Viral infections have been linked to diabetes mellitus since the turn of the 20th
century, when Harris described a patient who developed diabetes soon after infection
with mumps.[43] Coxsackie B virus, rubella and other viruses have varying levels of
evidence for association with type 1 diabetes.

Coxsackie B virus

Strong epidemiological evidence has accumulated for the role of Coxsackie B
virus (CBV) in the development in type 1 diabetes and some have suggested that the
evidence is now overwhelming. Initial studies found coxsackie B virus infection more
frequently in recently diagnosed cases of diabetes, suggesting a possible role in the
precipitation of clinical disease.[8] As more studies were done, it was found that the virus
can infect β-cells directly and has been isolated as a cause of fulminant pancreatic
infection.[36] Later, prospective studies, have shown an increase in infection before
development of disease, including infection in the prenatal period, indicating that
coxsackie B virus may play a role earlier and more important than precipitation of
disease.[8] In mice, infection of genetically susceptible strains with this virus results in
pancreatic destruction, supporting the idea that CBV is more than a precipitator of
diabetes.[44]

The mechanism of this causality is not entirely clear. While CBV directly infects
pancreatic cells, it may also play a role in initiating immune dysfunction by more indirect
means. Coxsackie B Virus has homology with glutamic acid decarboxylase, one β cell
protein, and so molecular mimicry, discussed below, may be a factor. Both of these
mechanisms may be important in the epidemiological association found between CBV
and type 1 diabetes.[46, 47] One currently favored hypothesis is that repeated infection
with Coxsackie virus is responsible for progressive destruction of the pancreatic beta
cells.[8]

**Congenital rubella and others**

Other viruses, such as rubella and mumps have been shown to be related to type 1
diabetes, and the role of breast feeding and cow's milk has received much attention.
Twenty percent of children with congenital rubella, which has been largely eradicated,
are estimated to develop diabetes.[8, 9] Mumps has been implicated, with less compelling
evidence. Cow's milk and breast feeding have been heavily studied as risk and protective
factors, respectively, but there does not seem to be a strong association, if any.[48]
Certain uncommon drugs, such as streptozocin, are diabetogenic, but are important mostly in an experimental setting.[9]

**Cytomegalovirus - general**

In 1979, a case report of diabetes in a 13 month old child with congenital CMV was published.[43] Soon after, pancreatic islet cell infection with CMV was demonstrated.[49] In the years since then, numerous studies have been done on humans and animals examining the link between CMV and type 1 diabetes.[43] Cytomegalovirus is part of the herpes virus family, which includes the Herpes Simplex Viruses, Varicella Zoster virus, HHV-6, and Epstein Barr virus. It is a common virus, with 50-100% of the US population infected by adulthood.[11] The peak incidence of infection is in early childhood, and there is another peak in late adolescence. This pattern differs in people from lower socioeconomic status who tend to be infected at an earlier age. Virus is secreted in bodily secretions, including saliva, breast milk, tears, urine and feces. Most infections in children are probably a result of breast feeding, with sexual activity being a major mode of transmission in adults. [50]

Although the acute infection is almost always sub-clinical, CMV causes congenital abnormalities in children, mononucleosis in some immunocompetent adults, and pneumonia and other diseases in the immunocompromised.[11] It accounts for about 8% of cases of mononucleosis. [50] CMV mononucleosis is most common in women in their 20's, and it resembles EBV mononucleosis, with milder symptoms. Like EBV mononucleosis, the major symptoms are persistent fever, myalgia, tonsillitis, and cervical lymphadenopathy.[50]
Congenital infection causes preterm birth, thrombocytopenic purpura, splenomegaly, hepatomegaly, prolonged neonatal jaundice, pneumonitis, deafness, cerebral palsy, epilepsy, chorioretinitis, microcephaly, hydrocephalus, and mental retardation, among other things.[11] One out of five hundred infants born in the US are congenitally infected with CMV, making it more important than the genetic syndromes as a cause of mental retardation. Only 10% of those infected show clinical signs at birth, although a large percentage develop long term sequellae. In an attempt to avert these tragedies, vaccines are being researched, but have not yet shown success in eliciting immunity in volunteers.[51]

After primary infection, it is possible to detect virus in the blood for weeks to months.[50] White blood cells are major sites of latency following initial infection, but there may be other sites.[50] Antibodies are present indefinitely, but are not protective. In the control of CMV infection, cellular immunity is thought to be the most important, with the key cells being CD8+ and NK cells. Although CD4+ cells can control infection in some organs, it seems that CD8+ cells are the cells most responsible for CMV specific immunity. The cytokine TNF seems to be important in the immune response to CMV, but also probably mediates some of the pathology caused by CMV, including the rare complication, hepatitis.[50] Besides hepatitis, CMV is associated with other autoimmune syndromes. Guillain-Barre syndrome, hemolytic anemia and thromboctyopenia are all uncommon sequellae to CMV mononucleosis.[50] Recently, dilative cardiomyopathy has been linked to CMV infection, because higher levels of antibodies to CMV, as well as enteroviruses and S. pyogenes, were found in patients with this condition. Signs of a Th1 imbalance, such as high levels of IgG2 and IgG3, were found in these patients.[52] PCR
evidence of CMV infection has also been found in the autoimmune skin disease vitiligo[53], which is commonly found in conjunction with other autoimmune conditions such as autoimmune thyroiditis.[7] CMV has also been linked to another autoimmune disorder, Sjogren's syndrome[46], perhaps mediated by a defect in apoptosis causing immune cells to accumulate abnormally.

The effects of CMV on the immune system include both widespread activation and widespread dysregulation. During infection it has been shown that other viruses are reactivated, including rubella and measles. There is some depression of cell mediated immunity (delayed type hypersensitivity), possibly mediated by the inhibition of MHC I expression on infected cells, and also impairment of MHC class II antigen presentation.[50]. There is a diminished proliferation of lymphocytes in response to certain challenges, including concavalin A, phytohemagglutinin, and other antigens.[50]. There are abnormalities in secretion of IL-2 in T cells specific for CMV, and other impairments in activation of T cells. The activity of Natural Killer (NK) cells, which are main players in controlling CMV infection, is also inhibited by CMV.[50] Concurrently with those changes, there is a polyclonal B cell activation. These responses are normal, but it may be that some respond differently, and that pathologic sequelae develop after an abnormal immune response. The effects of CMV are varied, and involve immune deviation as well as direct pathology.

2 This property of MHC: inhibition is shared by other DNA viruses, including adenovirus, papovavirus and poxvirus, and some RNA viruses including paramyxoviruses, retroviruses, coronaviruses, and rhabdoviruses. (Scholz, 1998)
Evidence for association between CMV and type 1 diabetes

Human and animal studies of the relationship between CMV and type 1 diabetes have given conflicting results. For a more thorough examination of the human research about this link, see paper 1. Several animal studies have been done on the link between CMV and diabetes. While some have found that cytomegalovirus causes insulitis in laboratory animals[54], most of the work done suggests that CMV might work as an accelerator or co-factor with other environmental risks.[54, 55] A conclusive answer to the question of whether CMV causes diabetes is as elusive in animals as it is in humans.

Several authors have suggested that CMV works together with other environmental agents to cause diabetes. Onodera found that MCMV and Reovirus caused diabetes when given to mice in sequence, but not when either virus was given alone. He also found that streptozocin, a diabetogenic drug, caused diabetes at a much higher rate in those animals who were infected with MCMV earlier.[39] An extensive study of the effects of CMV on the murine pancreas found that in C57BL/6, NOD, BALB/C and BALB.B mice, pancreatitis was caused by MCMV infection, but diabetes did not develop.[55] These results do not mean that CMV is not associated with the development of diabetes; they only suggest that CMV does not act alone to cause diabetes. In humans, some have postulated that; "CMV may be contributing to trigger ICA production without determining the development of type I diabetes, ...suggest that other factors may act synergistically to CMV infection in inducing some cases of IDDM."[56] It may be that CMV and some other environmental factor act as a one-two punch that leads to functional destruction of the beta cells of the pancreas.
Autoimmune mechanisms

Several mechanisms for the association between autoimmune diabetes and viral infections, including CMV, have been proposed. Roughly, these mechanisms can be divided into two general categories. First, viruses can cause an exposure to self proteins that were hidden from the immune system before infection, or to foreign antigens homologous to self proteins, causing a specific proliferation of self reactive clones, or, second, they can work further downstream, causing a dysregulation of the immune response. The latter process is indirect; dysregulation can turn a non-pathologic, physiologic self-reactivity into a destructive autoimmune disease. [12, 22, 57] Of course, these divisions are oversimplified, and there may be overlap between mechanisms.

Molecular mimicry

Whether there is an immune response to a given antigen depends greatly on the way that the antigen is presented to the immune system. It has been shown that self-reactive antibodies are generated when normal human proteins are given subcutaneously to subjects. Antibodies to these proteins, which include human Factor VIII and human insulin, may or may not create functional disturbances, but provide a model for how autoimmunity can be induced.[58] An elegant experiment demonstrated the ability of viruses that shared "self" proteins to induce autoimmunity, and shows how molecular mimicry could cause a destructive reaction. In this experiment, a viral protein was introduced into transgenic mice under control of the insulin promoter, and did not cause destruction of the islets, although T cells specific for this protein were found in the mouse. When the mice were later infected with the virus, they developed diabetes. [59,
60] Although this situation is somewhat artificial - the protein was exactly homologous, and not a "normal" protein - it shows that the immune system can be induced by a virus to respond to a protein that it has ignored before. Of note in this experiment is the fact that central tolerance did not need to be broken in order for autoimmunity to occur. [59]

Molecular mimicry occurs when "antibodies or T cells generated in the response to an infectious agent cross-react with self antigens". [16] Molecular mimicry can be caused by as few as three to six shared amino acids in proteins that are hundreds of kDa long, [22, 61] and is believed to be a factor in several autoimmune diseases. Antibody mediated molecular mimicry is a cause of rheumatic fever, a delayed reaction to certain Streptococcus species. [16] Coxsackie B virus, discussed above, shares homology with a sequence of the GAD65 protein, and this has been implicated in its association with type 1 diabetes. [6, 62] Cytomegalovirus, too, has some homology with islet antigens, in particular with a GAD epitope. [63] Because of this some have argued for a mechanism of molecular mimicry in a relationship between CMV and type 1 diabetes. Simple homology does not prove molecular mimicry, and some have questioned whether there is any crossreactivity between these human antigens and CMV, or even coxsackie B virus. Ricter found no such crossreactivity between antibodies specific for GAD proteins and homologous CMV and coxsackie B virus proteins, bringing the whole idea of molecular mimicry causing diabetes into question. [64] T cell mediated molecular mimicry is even harder to prove because of the difficulties associated with determining the antigenic specificities of T cells. As of yet, no conclusive proof of any T cell mediated molecular mimicry has been achieved, much less of CMV molecular mimicry. [16]
Release of self-peptides

Although molecular mimicry relies on similarities between viral antigens presented to the immune system and self antigens, it is not the only way that viruses can cause sensitization to self. Direct infection of the beta cells of the pancreas could also cause the immune system to perceive host proteins in a new or different way. While self-antigens are not normally presented to immune cells with stimulatory signals, viral fragments are displayed by antigen presenting cells (APCs) in a context that causes activation. Self proteins can be presented as viral proteins in a number of ways. During viral replication, the virus incorporates host cell peptides into its envelope. When APCs display portions of the viral envelope they could also display these incorporated self antigens. Self-reactivity by this mechanism is hypothesized to occur in a multiple sclerosis like syndrome in mice caused by the Semliki Forest virus.[22] Infection could also cause damage to the cell, leading to abnormal or excessive presentation of self peptides that are usually hidden from immune surveillance. This is believed to be involved in the autoimmune destruction present in polymyositis and chronic viral hepatitis.[22]

In order for CMV to cause damage through these mechanisms, CMV would have to directly infect the beta cells of the pancreas. Whether CMV infection of the pancreas is an important phenomenon is up for debate. In one study, twenty out of 45 children who died of disseminated CMV disease had CMV inclusion bodies in their beta cells,[65] but there has not been evidence of CMV in the pancreases of newly diagnosed diabetics.[66–69] Cultured human fetal islets are susceptible to infection with CMV, but this infection does not lead to changes in insulin production.[70] In a diabetic rodent model, CMV has
been found in the pancreases of animals displaying insulitis[65], but in experimental mice, exocrine infection but not endocrine infection with CMV was found.[55] Thus it appears that the evidence in humans and animals is against those mechanisms that require direct infection of pancreatic islets.

*Polyclonal activation*

Paradoxically, CMV causes both generalized suppression and activation of the immune system, and both mechanisms may be factors in autoimmunity. A polyclonal B cell activation has been found during CMV infection, and increased levels of IgG, antinuclear factor and rheumatoid factor have all been attributed to this generalized proliferation.[50]. This effect is thought to be unrelated to direct infection with CMV because the proliferation involves many more cells than the number infected with CMV.[50]. The question then becomes; is the autoimmunity, if there is any, specific for islet cells, or is it the result of this generalized activation? To answer that question, one group looked at the relationship between antibodies to CMV and autoimmune antibodies, both pancreas specific and non-specific, in healthy siblings of diabetic patients. The purpose of this project was to see if CMV induced a non-specific polyclonal autoimmunity or if it was specific to the pancreas. They found an association between pancreatic antibodies (specifically ICA) and CMV antibodies only, and did not find an association with other antibodies, including anti-mitochondrial, anti-nuclear, liver kidney microsomal antigen, rough and smooth muscle, reticulin, ribosomal and gastric parietal cells.[56] In contrast, others have found multiple autoantibodies unrelated to the site of infection.[71]
It has been suggested that CMV might act as a superantigen. Superantigens activate T cells by binding to MHC molecules and the T cell receptor in an area outside of the antigen binding site. In this way, many more T cells are activated, in a manner non-specific for the particular antigen.[16] There is some evidence for the presence of superantigens in type 1 diabetes, including the increased presence of T cells with a certain part of the T cell receptor[72], and there is some evidence that CMV acts as a superantigen.[59, 73] Infection of white blood cells or activity as a superantigen are both plausible mechanisms for non-specific activation of immune cells.

Inhibition of protective lymphocytes

Perhaps more interesting is the role of immune suppression in autoimmunity. Several pieces of evidence point towards the importance of regulatory mechanisms in the control of islet cell destruction. Frank insulinitis develops slowly in the mouse and human, with diabetes becoming apparent only months to years after the start of infiltration. When lymphocytes isolated from the pancreases of mice suffering from end stage destruction are transferred to disease free inbred mice, diabetes develops quickly. This has been interpreted as evidence for the slow attrition of protective lymphocytes that keep the inflammation in check in the first mouse, so that by the time destruction is complete, destructive cells unaccompanied by regulators are transferred to the second mouse. [6, 28, 35, 44] Other evidence has supported this. Mice who are given only islet cell lymphocytes extracted from diabetic mice develop diabetes much faster than mice who are given a combination of islet and spleen lymphocytes.[74] The spleen, a harbor for lymphocytes, contains cells which regulate the destructive potential of the islet
lymphocytes. The loss of this activity may be necessary for complete destruction to occur. It has been suggested that cyclophosphamide, which accelerates diabetes in NOD mice, acts by depleting suppressor T cells.[44]

Other experiments have shown that lymphopenia, induced by thymectomy or by other means, induces organ specific autoimmunity. While it is possible that the lymphopenia makes the animals more susceptible to infection, or that there is a compensatory proliferation of peripheral lymphocytes, it is also possible that these procedures eliminate suppressor T cells.[75] Suppressor T cells, as discussed above, are CD4+ cells that are not fully understood. They, like all other T cells, are thymically derived, and may be antigen specific.[18]

CMV may work by this mechanism. It has been shown to inhibit protective T cell subsets, accelerating diabetes in susceptible mice.[55] It has also been found that MCMV causes thymic involution in BALB/c mice, resulting in 80-90% fewer thymic cells despite the fact that fewer than 10% of the cells in the thymus are infected with MCMV.[76].

In addition, type 1 diabetes is believed to involve a pathologic Th1 dominance. As discussed above, destruction of pancreatic islets has been halted in mice by antigenic challenge that shifts the cytokine balance from Th1 to Th2.[14] CMV infection has been association with Th1 responses in diabetes and other autoimmune diseases, and with diabetes in mice. MCMV hepatitis in BALB/C mice is associated with higher levels of Th1 cytokines, and CMV has also been found in a Th1 cardiomyopathy.[52] In mice, MCMV infection in conjunction with type 1 diabetes has been associated with Th1 cytokines.[55]
Thus, it seems that an interaction between CMV and immune regulators is one of the most likely mechanisms by which CMV could cause diabetes. More research is needed into this area, especially the relationship between cytokines and cell types, and the influence of CMV.

**Non-autoimmune mechanisms**

Besides immune mechanisms, there are a number of other possibilities that could explain an association between CMV and diabetes. The first to consider is that CMV could precipitate the clinical onset of diabetes in someone who already has extensive beta cell damage and brittle glucose control. Infection in general is associated with the release of catecholamines and glucagon, antagonists of insulin,[77] and so causes metabolic stress. In a patient with few functioning beta cells, CMV or another infection could be the proverbial straw of increased insulin demand breaking the camel's back of glucose control. However, in the meta-analysis of human research that accompanies this paper, no evidence was found for increased levels of serologic markers of recent disease in newly diagnosed patients with type 1 diabetes.[56, 78-81]

Similarly, CMV could be reactivated by the onset of diabetes, so that it could be the evidence of infection rather than infection itself that is related to diabetes. (ref) It is difficult to determine whether a CMV infection is primary or a reactivation. Reactivation causes an IgM response, similar to a primary infection.[50] If reactivation is a consideration, prospective studies would be needed to sort out when initial infection with CMV occurred. Because there was not evidence of increased CMV IgM in diabetic patients, this possibility seems less likely.
Another possibility to consider is that CMV could facilitate other infections. Already discussed above was the fact that CMV causes widespread immune suppression, and infection with CMV could increase susceptibility to the "true" etiologic agent. Analysis of CMV and risk of diabetes should also include analysis of the other plausible infectious agents.

The possibility of confounding should never be overlooked. The direction of bias could be in either direction. Because childhood infection is often caused by breastfeeding, any protective effect of breastfeeding on diabetes would confound the relationship between type 1 diabetes and cytomegalovirus, giving a bias towards the null if breastfeeding is protective. Socio-economic status, other infections, and underlying immune problems could also confound the association between CMV and type 1 diabetes.

**Conclusion**

Much remains unknown about the etiology of type 1 diabetes. Although some infections have been tied to diabetes in mouse models, NOD mice develop diabetes at a much higher rate when they are kept in sterile environments than when they are exposed to the normal mice pathogens. The link between this tendency and a similar one in humans has not been made.[30] This may reflect a different disease pathology in humans and NOD mice, or an unexplored area of human disease. Asthma and other allergies have been associated with the lack of certain childhood infections, indicating that the immune system needs to be stimulated in order to curb abnormal reactivity. In addition, some infections are more severe when they occur in an older host, including the herpes viruses Epstein Barr virus and varicella. The current evidence linking coxsackie B Virus and
rubella to type 1 diabetes is not consistent with the idea that lack of infection leads to diabetes, and this may reflect a major difference between humans and this animal model. However, the way that we currently think about the immune system is certainly overly simplistic. A more flexible paradigm that views the immune system as a dynamic, balanced set of interacting forces is needed. We do not know if we are depriving the immune system of critical challenges without which proper development does not occur. The past centuries have seen unprecedented social change in terms of hygiene, migration and our relationship to the environment. The effects of these changes have yet to be seen.

Clearly the relationship between CMV and diabetes is complicated. It is still not clear by which mechanism, or mechanisms—if any—CMV works to cause type 1 diabetes. Molecular mimicry, inhibition of protective cells and polyclonal activation are all possible, although there is no definitive evidence for any one. As our understanding of the subtleties of the immune system has progressed, our appreciation how intricate the relationships are has also grown. As much as we might desire simple answers, it is unlikely that any will be forthcoming.
References


4. Tuomilehto, Epidemiology of childhood diabetes in Finland-background of a nationwide study of Type 1 (insulin dependent) diabetes mellitus. Diabetologia, 1992. 35: p. 70-76.


27


Meta-Analysis

Background and Significance:

Type 1 Diabetes Mellitus is the most common chronic disease of childhood, affecting 1.3 million Americans.[1, 2] Despite intensive research in the past twenty years, it is still unclear what factors are important in the etiology of this disease. Cytomegalovirus (CMV), a common virus, is a particularly controversial factor because of the conflicting evidence about its role.

Although the availability of cheap and effective insulin therapy has greatly decreased the morbidity and mortality associated with type 1 diabetes (T1D), it continues be a major killer. Patients with diabetes have a four times increased risk of myocardial infarction, a twenty times risk of gangrene, and 15-40% likelihood of developing end stage renal disease. In addition, retinopathies, neuropathies and vascular disease can be disabling and life threatening. [1, 3, 4]

Long before a child presents with the hyperglycemia and ketoacidosis that are typical of type 1 diabetes, a slow destruction of pancreatic islets occurs. This process, insulitis, is characterized by lymphocytic infiltration of the islet region of the pancreas. Eventually, this infiltration leads to complete destruction of the islet cells that produce insulin and an inability to regulate glucose levels. Although the autoantibodies that accompany this syndrome include antibodies to islet cells, insulin, glutamic acid decarboxylase, and protein tyrosine phosphatase, it appears that the T cells are the true culprits.[5] Cytotoxic T cells are a large component of the inflammatory infiltrate, and, in animals, can be transferred to a normal subject and cause disease.[6] Islet cell antibodies are markers of the destructive process, though, and can be a surrogate endpoint in studies
of type I diabetes.[7] While it is generally agreed that this is an autoimmune disease characterized by T lymphocytes specific for beta cells, an imbalance of the immune response, and auto-antibody markers, it is still unclear which events and factors initiate destruction of cells, accelerate progression, and precipitate clinical symptoms.

Current theory postulates this disease requires both genetic susceptibility and multiple environmental insults in order for complete beta cell destruction to occur.[8] An identical twin of a type 1 diabetic has a 50-70% risk of developing the disease himself.[1] This indicates that inheritance may be necessary for disease, but it is not in itself sufficient. Environmental insults may serve as initiators of destruction, or by accelerating damage during the long inflammatory period.[5] Further, clinical symptoms may be triggered by an insult that is unrelated to the ongoing insulitis.

Environmental factors from cow's milk to viruses such as mumps, rubella, coxsackie B and CMV have been investigated as etiologic agents with varying degrees of success. Rubella is strongly associated, with an estimated 20% of congenitally infected children going on to develop type 1 diabetes, and mumps has been suggested.[9] Rubella and mumps are now rare diseases and are unlikely to account for the bulk of type 1 diabetes.[10] Coxsackie B virus is also strongly associated epidemiologically, and animal studies have demonstrated a causal role for CBV in type 1 diabetes.[5, 11]

Cytomegalovirus was identified as a potential factor following the case report of a child with congenital CMV who developed diabetes.[12] Soon after, it was demonstrated that CMV can infect pancreatic islets during disseminated infection[13], and human and animal studies began to be published corroborating and questioning the connection between CMV and diabetes.
Cytomegalovirus is a common herpes virus that causes a variety of diseases. By adulthood, over 50% of people have been infected in the US, with higher levels of infection among lower socio-economic groups. The peak incidence of infection is in early childhood, and there is another peak in late adolescence. The virus remains latent in lymphocytes after infection. The acute infection is almost always sub clinical, but causes mononucleosis in some immunocompetent adults, and causes a variety of diseases in immunocompromised patients.\[14\] Like Rubella, another disease associated with type 1 diabetes, CMV can cause serious congenital abnormalities, and is the most common cause of mental retardation in children.\[15\] Congenital infection may be particularly important because it may be that during the prenatal period the developing body is especially susceptible to immune dysregulation.

Animal studies have suggested that insulitis in genetically susceptible mice is accelerated by infection with a murine cytomegalovirus. One study found that CMV inhibits certain T cell types that might be protective against diabetes.\[16\] This concurs with other work that shows that peripheral lymphocytes are protective against pancreatic destruction in the mouse.\[17\] Other mechanisms, such as molecular mimicry, direct infection, immune deviation, and super antigens have been proposed.\[16\]

The studies that have been done in humans on the link between CMV and Type I diabetes have shown inconsistent results with little agreement about the role of CMV in the development of diabetes. This paper examines the literature and analyzes it within a meta-analytic framework to determine whether a summary answer can be found to the question: "Is CMV associated with the development of type 1 diabetes?". Trends are also
analyzed that may explain the heterogeneity of the findings presented in the published literature.

In addition to clinical diabetes, the relationship between CMV and islet cell antibodies was also evaluated. An estimated 50% percent of children with islet cell antibodies (ICA) go on to develop type 1 Diabetes [1], although this varies by study and age.[7, 18] Studies addressing the relationship between CMV and ICA were also analyzed.

Methods

Study selection

Medline (1966-July 2000), the Melvyl database BIOSIS, and Doctoral Dissertation Abstracts On-Line (North American Universities, 1861-2000) were searched with the keywords and MESH headings given in Table 1. Major diabetes conference abstracts for 1991-1999 were searched by hand.[19-25] Titles and abstracts of the studies obtained by these methods were scanned, and those that seemed relevant were obtained. The secondary references of these papers were scanned and relevant references were obtained. Spanish language articles were translated by the author, Chinese and French articles were translated by others.

Studies were included for abstraction if cases were defined as type 1 diabetics or were islet cell antibody positive, the studies assessed CMV exposure and had controls.

Data abstraction

The following data were abstracted onto a standardized form: study type, ages of subjects, source of controls, years of the study, method of obtaining exposure
information, type of outcome measures, the method of diagnosis, the time from onset of diabetes to data collection, the country where the study was conducted, and the raw data used to compute risk ratios. Inclusion criteria included those mentioned above and the requirements that: CMV exposure be measured no more than one year after onset of type 1 diabetes, that controls must not be specifically ICA positive, a marker of a pre-diabetic period, and that an odds ratio be calculable from the data. When data was redundant between studies, the study that presented more complete or more recent data was chosen.

Statistical analysis

Analysis was complicated by the presence of sparse data in some categories within certain studies. Although asymptotic methods of calculating odds ratios are easily done by hand, they require assumptions about the distribution of parameter estimates that were not valid in this case. Exact methods, although more computationally difficult, allow for calculation when sparse frequencies are present, relying only on the distribution of the data. Because assumptions of large sample size and expected frequencies were not met, exact methods were used. Statistical analysis was done using the program EXACTMA, written by Martin and Austin for meta-analysis, and available at: http://www.sph.emory.edu/~haustin/exactma.html. A full description of EXACTMA may be found in reference 25.[26] Results of these analyses were very similar to Mantel-Haenzel approximate methods, with the exception of the tests for homogeneity, where approximate methods consistently underestimated exact heterogeneity.[27] For all analyses in this paper, Mantel-Haenzel exact methods are used.
Assigning quality scores is one weighting method that has been used in meta-analysis. This may introduce added bias, particularly when there is no objective way to assess quality, without adequately adjusting for differences between studies. Quality scoring has not been shown to improve the results of meta-analysis[27], and was not possible when using EXACTMA. For these reasons, quality scoring was not performed.

Because the majority of islet cell destruction is hypothesized to occur months or years before the onset of diabetic symptoms, the exposure estimate that reflected earliest infection with CMV was chosen for studies that reported data for more than one exposure. While studies measuring IgM or IgG as indicators of infection were both included in sub-analyses, IgG is theoretically a better marker for the exposure of interest here. IgG antibodies represent past infection and persist indefinitely after initial CMV infection, while IgM antibodies reflect recent infection or reactivation and are usually present in the initial stages of infection. Data concerning IgG antibodies to CMV was thus chosen in preference to data concerning IgM antibodies for the overall analysis. For one study it was necessary to choose between congenital exposure and later antibodies (both IgM and IgG). Because the study that measured antibodies measured CMV infection more directly than the study that measured maternal antibodies, it was chosen in preference.

Besides the overall analysis, subanalyses by exposure and outcome type were done to assess sources of heterogeneity. Subanalyses were done for studies that measured IgG CMV serology, IgM CMV serology, DNA CMV analysis, and congenital CMV, as well as for those studies that measured islet cell antibodies instead of diabetes as an outcome.
Results

Thirty-five potentially relevant studies were identified. Twenty-six came from the Medline search described above. Six were identified by manual searching of the references of the initial studies identified, and three were identified on a search of the database BIOSIS. Seven of the thirty-seven studies fulfilled the final inclusion criteria.[28-34] The excluded studies and the reasons for exclusion are listed in Table 2. The most common reason for exclusion was that the study used neither type 1 diabetes nor islet cell positivity as an endpoint. Other common reasons for exclusion included lack of a control group, lack of CMV measurements, data redundant with other studies, and too long of a time between onset of diabetes and measurement of CMV exposure. A summary of the seven studies included in the meta-analysis appears in Table 3. Five studies were included in the overall analysis, and the other two were used in subsequent analyses that included studies that measured islet cell antibodies as an outcome. There is considerable variation in the way that exposure to CMV was measured in the studies included. Measurement of exposure ranged from serologic assessment of IgG and IgM antibodies[28-30, 33, 34], PCR or other DNA analysis of lymphocytes[32, 33], and measures of congenital exposure.[32, 33] This variety of measurements represents quite different types of exposure, indicating possible sources of heterogeneity.

In two additional studies, islet cell antibodies, which are predictors of pancreatic damage as discussed above, were used instead of clinical diabetes to define cases [28, 31].
TABLE OF INCLUDED STUDIES

<table>
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<tr>
<th>Serology</th>
<th>#Study Design</th>
<th>Exposure Type</th>
<th>#Cases / #Controls</th>
<th>†Baseline prevalence</th>
<th>Age range cases</th>
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<td></td>
<td></td>
<td></td>
<td>IgG IgM</td>
<td></td>
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<td>&lt;7</td>
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<td>Outcome: Islet Cell Autoantibodies ICA</td>
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<td>44/44</td>
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<td>12.5+/8.4</td>
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<td>Ivarsson 1993 [32]</td>
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<td></td>
<td>19,556*</td>
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<td></td>
<td>Maternal IgM in 1st trimester</td>
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†Baseline prevalence calculated from CMV prevalence in control group.
*Refers to total size of cross sectional and cohort studies.
ϕCC=case control, CO=cohort, XS=cross sectional

Table 3.

Principal analyses and subanalyses

Overall

An analysis was done with all of the studies that used diabetes as an endpoint to test the hypothesis that CMV was associated with elevated risk of developing diabetes. The five studies eligible for this analysis had relative risks that ranged from 0.46 to 10.46, with four greater than and one less than 1 [29, 30, 32-34]. Only one study achieved statistical significance in isolation[30]. The overall analysis found evidence of statistical heterogeneity (Mantel Haenzel test for homogeneity: p=0.06631), using a conservative p
value of 0.1 or lower to indicate heterogeneity, and a summary estimate of 1.635 (95% CI 0.9938-2.688, p=0.05256). See figure 1. This evidence of heterogeneity could mean that the studies are not a random sample of a literature with the same underlying risk ratio, and a summary estimate of such a literature is generally not regarded as valid. This result suggests that the studies vary in more substantial ways than simply random variability, and that reasons for that heterogeneity need to be explored.

Stratified analyses:

Stratified analyses based on exposure type were performed to evaluate potential sources of heterogeneity. See figure 2.

**IgG**

Two studies assessed IgG serology in newly diagnosed diabetics[29, 33], and another two studies assessed IgG serology in the presence of ICA[28, 31]. Analysis of the two diabetes studies did not show evidence of heterogeneity (P = 0.55692). The summary estimate for these two studies was not significant (RR: 1.272 CI: 0.7297, 2.216).

With the two studies that used ICA as an endpoint instead of clinical diabetes included, homogeneity is lost (P = 0.04161). The summary odds ratio was 1.79 (CI: 0.9930, 3.221).

**IgM**

One study assessed IgM serology and diabetes[33], and two studies assessed IgM and ICA[28, 31]. When these three studies were combined, a summary estimate did not
demonstrate significant underlying heterogeneity (P = 0.27520). The summary odds ratio did not differ significantly from 1 (RR: 0.6565 CI: 0.1787-2.412).

PCR

Two studies used analysis of viral DNA in lymphocytes as an exposure marker for CMV[30, 34]. When these two studies are analyzed, an odds ratio of 4.7 is found (CI: 1.13-19.71), but these studies are not statistically homogeneous (test for homogeneity P=.04).

Congenital CMV

Two studies were prospective analyses of the risk of diabetes associated with congenital CMV infection.[32, 33] One study assessed congenital CMV by looking at the IgM antibodies of mothers in their first trimester.[33] This may not be an adequate measure of true CMV infection in utero. These two studies did not demonstrate statistical heterogeneity (p=.25), and the risk ratio was not significant at 2.22 (CI: .49-9.98).

Overall with ICA

Islet cell antibodies appear before clinical symptoms of diabetes begin, and predict eventual disease about 50% of the time. Two studies looked at the association between ICA and CMV IgM and IgG. If these studies are included in the overall analysis, significant heterogeneity between studies was found (MH test for homogeneity p=0.00493). The summary risk ratio estimate was 2.385 (CI: 1.382-4.118). An analysis of these two studies alone also yielded considerable heterogeneity (P = 0.00036), and a relative risk of 2.014 (CI: 0.9966-4.071).
Sensitivity analyses

The overall estimate of 1.635 is sensitive to the inclusion of the study by Hiltunen et al.. With removal of this study, the RR is 3.744 (1.369, 10.24), and the MH test for homogeneity is not significant (P = 0.18136). This study published several different arms with different study designs. The design included here was a case-control study that measured titers of CMV antibodies in children who were diagnoses with type 1 diabetes before the age of 7. This study arm was chosen in preference to the others because one of the other arms used ICA positive individuals as controls, while the other measured CMV serology from the mothers of cases and controls during pregnancy. This is an indirect measure, at best, of CMV infection in the diabetic children and controls. Otherwise, the analysis was not sensitive to the inclusion of the remaining four studies.

Publication bias

![Figure 2. Funnel Plot](image-url)
Although no abstracts were found relating to CMV and type 1 diabetes that were not published in later papers, there is some reason for concern about publication bias in this study. A plot of risk ratio estimate size against sample size for these studies does not give the expected "funnel" shape. See figure 2. Further, one study which was excluded because it did not report results about diabetes and did not have a control group assessed the long term health effects of viral infections in utero. One would assume that if this study had found a high incidence of diabetes in the CMV infected group, this result would have been published. On the other hand, the Guo study which was rejected because Type 1 and 2 diabetics were mixed, found an association between CMV and diabetes. [35] The possibility of publication bias can not be ruled out.

Discussion:

When the available literature is summarized, a trend towards an association between CMV and type 1 diabetes is found, although it appears that there may be heterogeneity in the literature that cannot be explained by random variation alone. Understanding the sources of that heterogeneity is valuable in understanding the state of the literature and directions for further research. Much of this heterogeneity is due to differences in exposure measurements between studies. When stratified by exposure, all sub-groups except for the studies doing CMV DNA analysis were internally homogeneous. However, in these sub-analyses, no statistically significant association between CMV and T1D was found, perhaps because the strata were small and remained under-powered. What is clear from the results is that recent CMV exposure, as measured by IgM, is not likely to be associated with T1D. Other measurements, all reflecting
exposure of longer duration, found positive results that were not quite significant at a 95% confidence interval, but showed a definite trend towards a positive association. The results of the individual sub-analyses can help inform our understanding of the possible association between CMV and type 1 diabetes.

As discussed above, viruses are hypothesized to act as initiators of beta cell destruction, accelerators and as precipitants of clinical symptoms of diabetes. In order to parse out the role of CMV, it is necessary to understand the temporal association between CMV and diabetes. There are two ways to examine the temporal relationship between CMV and diabetes in the literature presented here. The first way is by looking at the differences between risk ratios based on IgM and IgG antibodies. If measurements of IgM were highly correlated with recent diagnosis of diabetes, it would be probable that CMV acts as a precipitator of diabetic symptoms. Although some have argued for this mechanism of viral interaction with diabetes, my results do not support this theory. Instead, my results suggest that CMV is not an immediate precipitator of diabetes. Nor does it appear that diabetic onset makes one more susceptible to new infection with CMV, another suggested possibility with regards to viruses.[5, 8, 33, 34] Studies that did not meet inclusion criteria support these findings; both Bantavala and Pagano found no CMV IgM in diabetic subjects. [36, 37]

The second way to examine the temporal relationship is by looking at the results of analysis of islet cell antibodies and CMV. Islet cell antibodies are markers of an ongoing inflammatory process in the pancreas. Therefore if CMV works by accelerating beta cell destruction after destruction has already begun, or by precipitating symptoms of diabetes, it would be expected that studies measuring an association between CMV and
diabetes would find a stronger association than those measuring an association between CMV and ICA. It was found that even the two studies measuring ICA and IgG levels had an unacceptably high level of heterogeneity (P = 0.00036), and analysis of IgM and ICA could not be carried out because of sparseness of cells. Inclusion of these two studies in the overall analysis also increased the overall heterogeneity to an unacceptable amount, leading to the conclusion that CMV and diabetes and CMV and ICA may not share a common underlying relative risk. It is not currently possible to assess the relationship between ICA and CMV. In recent years it has been reported that other autoantibodies such as GAD, IA-2 and insulin have a much higher predictive ability than ICA[38], and the relationship between these antibodies and CMV should be explored. More research needs to be done in this area to determine the true relationship between auto-antibodies and CMV.

Several possible ways that CMV could cause an increased risk of Type 1 Diabetes have been studied. Molecular mimicry, superantigens, polyclonal activation, inhibition of suppressor mechanisms and direct destruction are all mechanisms that have been proposed. From the human research done so far, it seems that direct destruction or local bystander effects are not likely pathologic mechanisms, unless all virally infected cells were destroyed by the time diabetes manifested and sampling was performed. Three studies assessed pancreatic infection but did not meet inclusion criteria. No CMV was found in the pancreases of diabetic subjects in any study; two of the studies found no CMV in the pancreases of controls, and the third study did not look at controls.[39-41]. Despite the publication of case reports that describe pancreatic infection with CMV in diabetic children[13], chronic infection of the pancreas does not seem to be a significant
process in either diabetic patients or healthy controls. With regards to the other mechanisms, it is not possible from these analyses to determine whether one mechanism or another is more likely.

Research on a certain strain of mice (NIH Swiss mice) has shown that CMV in association with other viruses, but not alone, causes diabetes.[42] Possibly, CMV works in conjunction with other factors to cause islet cell inflammation. Cytomegalovirus infection is known to cause immune dysregulation[15, 43], and this could facilitate other infections. Analysis of CMV and risk of diabetes should also include analysis of the other plausible infectious agents, to see if there is an interaction between this virus and other infections.

Besides statistical power, there are several other reasons that could explain why a spuriously weak relationship may have been found. Even if CMV were a causal factor in T1D, a relatively weak relationship could be explained by the fact that only a small percentage of the population is genetically susceptible to this autoimmune disease. Only one study used sibling controls[31], who would likely share some of the same genetic risk as cases. Although controlling more for genetic risk, this study design is not ideal because siblings do not have identical genetic risks, and CMV exposure is likely common to a family. An ideal study would measure genetic risk directly, through HLA typing and evaluation of other genes that increase risk.

Several of the studies included measured all subjects at age 7 or less, which is younger than the peak incidence of type 1 diabetes. Diabetes at an early age may differ from diabetes that develops at the more common age of puberty. Studying younger
children could bias the findings in either direction, and future studies should be carried out at the peak age of diabetic onset.

Like all meta-analyses, this analysis is limited by the quality of the studies published. Many of the studies published in this area were not designed specifically to assess the importance of CMV in type 1 diabetes. Several of them were either general follow-up studies of congenitally infected infants, or they assessed multiple viral and other environmental risk factors in newly diagnosed type 1 diabetics. Not only does this mean that studies were not designed to optimally answer this question, but is also indicates the real possibility of publication bias. In the studies presented here, four of seven studies were published with only results from CMV; three of these studies had risk ratios above 7, while the other three that published CMV results in combination with other results showed two negative results and one that had a risk ratio of 2.1. It may be that researchers are more likely to publish negative results if they are accompanied by other results than they are to publish negative results alone.

The low prevalence of CMV in these studies also introduced problems with analysis. Two studies were excluded from analysis because they reported no CMV exposure in either cases or controls, making it impossible to analyze the data. It is difficult to interpret data that shows neither case nor control group infected at all with CMV. Perhaps such studies were performed in a population with extremely low risk of CMV, or perhaps, as may be the case in those studies that used PCR, the methods of assessing exposure were inadequate to truly assess infection history.

There are limitations to what inferences can be made about causality from observational studies such as those included here. Factors that would usually support a
causal hypothesis such as dose response are not available in this analysis. Not only must we consider the usual caveats about confusing association with causation, but we must also remember that the temporal relationship between CMV and type 1 diabetes has yet to be established. Without knowing the sequence of events, it is impossible to know whether CMV is causally related to type 1 diabetes.

Confounding could also bias the results in either direction. Because childhood infection is often caused by breastfeeding, any protective effect of breast feeding on diabetes would confound the relationship between type 1 diabetes and cytomegalovirus, giving a bias towards the null if breast feeding is protective. Socio-economic status, other infections, and underlying immune problems could also confound the association between CMV and type 1 diabetes.

**Directions for further research:**

In order to know conclusively whether infection with CMV is associated with type 1 diabetes a prospective study that periodically measures CMV sero-positivity while also assessing predictive autoantibodies and clinical status should be conducted. Such a study would ideally be done from birth to the peak ages of diabetic incidence, 11-13. Using an increased risk due to CMV of approximately 2, as calculated from this analysis, and assuming that thirty percent of subjects are infected with CMV by age 11, a cohort of 31,614 people would be needed to power (beta=0.2, alpha=0.05) this investigation. Ideally, genetic risk, the contribution of other viruses, and confounding factors would also be assessed in this study. Currently, a study such as that described is being performed in Finland, but for the purpose of assessing the contribution of enterovirus
infection to diabetes. This study is an opportunity to also assess the relationship between CMV and diabetes, and CMV serology should be obtained if it is not already being done. In light of the size of the study needed, it is not surprising that the studies thus far performed have shown inconclusive results. Obviously, an investigation of this size would come at a considerable expense, and would require years to complete. Without knowing the temporality of CMV infection that would be provided by such a study, however, it will remain difficult to assess the relationship between CMV and type 1 diabetes.
### Tables and Figures

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Table 1.
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Table 2.
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**Summary**  
**P=0.07**  

*1.64 (0.99-2.69)*

* Refers to MH test for Heterogeneity

Figure 1.
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<th>Sub-analyses</th>
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*Refers to MH test for homogeneity

Figure 2.
References:


54


Tuomilehto, Epidemiology of childhood diabetes in Finland-background of a nationwide study of Type 1 (insulin dependent) diabetes mellitus. Diabetologia, 1992. 35: p. 70-76.
Conclusion:

Neither animal studies nor human studies provide a conclusive answer to the question of whether cytomegalovirus is associated with type 1 diabetes. Evidence from both suggests an association, but the magnitude and nature of that association remain to be determined. More work is needed both in evaluating the epidemiological association between this virus and diabetes, and in elucidating the basic mechanisms of autoimmune disease.
References For Introduction and Conclusion

