The Role of Viruses, Bacteria, and *Mycobacterium Avium* Subspecies *Paratuberculosis* Infection in the Etiology of Diabetes Mellitus

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1 Pathogens and Diabetes

Convincing evidence to date indicates that microorganisms are associated with Type 1 diabetes (T1D) development and progression. Insulin-dependent T1D is a prototypic organ-specific autoimmune disease resulting from the selective destruction of insulin-secreting β cells within pancreatic islets of Langerhans by an immune-mediated inflammation involving autoreactive CD4+ and CD8+ T lymphocytes which infiltrate pancreatic islets.

T1D is a paradigmatic example of chronic multi-factorial disease determined by the interaction of genetic, environmental and immunologic factors with consequent destruction or damaging of the β-cells in the islets of Langerhans, insulin deficiency and hyperglycemia (Coppieters et al., 2012). T1D is a polygenic disease, in which the genetic background is essential but not sufficient in causing the disease (Patterson et al., 2009). Indeed, approximately 85-90% of new onset T1D patients do not have a first degree relative with the disease, this implies a strong environmental component to contribute to the development of T1D (Coppieters et al., 2012). While several genetic susceptibility loci have been pinpointed by genome-wide association studies (Concannon et al., 2009), the environmental factors at play remain boldly elusive. Yet, environmental factors play a prominent role in T1D pathogenesis, as suggested by the incomplete (~65%) T1D concordance between monozygotic twins (Redondo et al., 2009), by migrant studies (Bodansky et al., 1992; Kondrashova et al., 2007) or by the decreasing weight of susceptible and protective HLA Class II haplotypes over the last decades (Hermann et al., 2003; Gillespie et al., 2004).

One of the environmental risk factors identified by a series of independent studies is represented by pathogens, with strong evidence showing that viruses and bacteria can infect pancreatic beta cells with consequent effects ranging from functional damage to cell death. Many experimental studies support the potential participation of viral infections to T1D pathogenesis, with a particular emphasis on virus-triggered islet inflammation, beta cell dysfunction and autoimmunity. Evidence from animal models suggests that viruses can trigger T1D in some cases. Viruses have also been implicated as possible triggers of other autoimmune disorders, including multiple sclerosis, autoimmune chronic active hepatitis, Sjögren's syndrome, juvenile rheumatoid arthritis and systemic lupus erythematosus (Schattner & Rager-Zisman, 1990; Talal et al., 1992). Several mechanisms have been proposed for virus-mediated autoimmunity.

First, a virus could alter the target tissue of the host such that the tissue becomes recognized as foreign by the host's immune system, thus triggering an autoimmune response. These alterations could include modification of surface antigens into immunogenic forms, induction of new antigens, release of sequestered antigens during host cell lysis, incorporation of cellular antigens with viral envelope, or up-regulation of MHC class I and/or class II molecules on the target tissue of the host. Second, the virus could alter the immune system of the host, resulting in autoimmune attack of beta cells. These alterations could include: - polyclonal B cell activation leading to production of autoantibodies, - release of lymphokines such as interferon (IFN)-α and tumor necrosis factor (TNF), which in turn recruit immunocytes to the host's tissues, - activation of immune cells that results in a breakdown of immune tolerance, or - disruption of the Th1/Th2 immune balance. Third, antigenic epitopes on the virus could be similar to molecules on the host tissue (molecular mimicry), thus causing the generation of antigen-specific T effector cells and/or antibodies that recognize the host target cell, leading to the development of autoimmunity. Fourth, it has been proposed that antiviral antibodies arising as a result of viral infection could lead to the formation of anti-idiotypic antibodies. These secondary antibodies could be autoreactive if the first antibody was produced against the part of the virus that reacts with the host (Jun & Yoon, 2003).
1.1 Viral Infections

Might persistent viral populations play a role in human T1D?

Viruses have long been suspected to contribute to the onset of T1D, based on detection frequencies around clinical onset in patients and their ability to rapidly trigger hyperglycaemia in the non-obese diabetic (NOD) mouse. Figure 1 shows a point–counterpoint type of approach about deciphering the etiology of human T1D (Coppieters et al., 2012).

The earliest observations were that the onset of T1D sometimes followed acute infections (Harris, 1898) and occurred with greater frequency at certain times of the year (Adams, 1926; Gamble & Taylor, 1969) which often indicates a viral cause. Then, epidemiological studies have shown the presence of virus-specific IgM antibodies in recent-onset T1D patients (Banatvala et al., 1985; Friman et al., 1985; Szopa et al., 1993). The most convincing evidence comes from studies in which viruses isolated from the pancreas of patients that died from acute T1D caused diabetes in animals by the destruction of beta cells (Yoon et al., 1979; Champsaur et al., 1982).

The most well studied environmental factor and the most robust association with viruses and T1D involves Enterovirus species, of which some strains have the ability to induce or accelerate disease in an-
imal models (Tracy et al., 2010; Getts & Miller, 2010; Stene & Rewers, 2012). A possible link was first reported by Gamble et al. (1969) with many subsequent studies, in humans and animal models of diabetes, showing an association, particularly with Coxsackie virus B-4. Higher rates of Enterovirus infection, defined by detection of enterovirus IgM or IgG, or both, viral RNA with reverse transcription polymerase chain reaction (RT PCR), and viral capsid protein, have been found in patients with diabetes at diagnosis compared with controls (Andreoletti et al., 1997; Lönnrot et al., 2000; Craig et al., 2003; Sarmiento et al., 2007; Richardson et al., 2009). Prospective studies have also shown more enterovirus infections in children who developed islet autoantibodies or subsequent diabetes, or both; as well as a temporal relation between infection and autoimmunity (Hyöty et al., 1995; Hiltunen et al., 1997; Lönnrot et al., 2000; Salminen et al., 2003). The extreme difficulty in biopsying pancreas has made it almost impossible to assay for viruses (or any other pathogen) in the pancreas at the time of T1D onset, a scientifically sound type of observation for associating specific pathogens with a disease. Associations of viruses other than Enterovirus with a T1D etiology (e.g. Rubella virus) (Menser et al., 1978) or in mouse models (Oldstone, 1988; Wilberz et al., 1991), as well as diverse reports of involvement of different Human Enteroviruses (HEV) in T1D onset (Tracy et al., 2010; Okada et al., 2010), continues to fuel debate as to either a specific role for diverse viruses in T1D onset or a role for specific viruses themselves. Further confounding the issue are data from the non-obese diabetic (NOD) mouse model showing that Human Enteroviruses can induce long-term protection from the onset of host-driven autoimmune T1D onset (Tracy et al., 2002; Tracy et al., 2010; Chatenoud et al., 2010).

Over a half-dozen human viruses have been reported to be associated with human T1D. These include Coxsackie B virus (Jensen et al., 1980; Yoon & Kominek, 1996), Rubella virus (Ginsberg-Fellner et al., 1984; Ginsberg-Fellner et al., 1986), Mumps virus (Gamble, 1980; Helmke et al., 1980), Cytomegalovirus (Ward et al., 1979; Pak et al., 1988; Yasumoto et al., 1992), Epstein-Barr virus (EBV) (Chikazawa et al., 1985; Surcel et al., 1988), Varicella zoster virus (Jali et al., 1990), Retrovirus (Conrad et al., 1997; Stauffer et al., 2001) and Rotavirus (Honeyman et al., 2000). The finding that Saffold viruses, a relatively recently characterized group of human cardioviruses (Chiu et al., 2008), are apparently widespread in the human population (Zoll et al., 2009) alters this dynamic and raises the question: could there be other viruses associated more commonly and more specifically with human T1D than HEV? It will be worthwhile to explore this topic, perhaps initially by utilizing microarray tools (Wang et al., 2002) which can assay for diverse species of viruses within a single sample.

About ten viruses have been reported to be associated with the development of T1D in animals. These include Encephalomyocarditis virus (EMCV) (Craighead et al., 1968; Yoon J et al., 1980), Mengovirus (Yoon et al., 1984), Reovirus (Onodera et al., 1978) and Retrovirus (Suenaga & Yoon, 1988; Gaskins et al., 1992; Nakagawa et al., 1992) in mice; Coxsackie B virus, particularly B4, in mice (Yoon et al., 1978; Hou et al., 1993) and nonhuman primates (Yoon et al., 1986); Foot-and-Mouth Disease virus in pigs and cattle (Barboni et al., 1966); Rubella virus in hamsters and rabbits (Menser et al., 1978; Rayfield et al., 1986); Bovine viral diarrhea virus in cattle (Tajima et al., 1992) and Kilham’s rat virus in rats (Guberski et al., 1991). As well as triggering beta cell-specific autoimmunity, three picornaviruses are may cause T1D by directly infecting and destroying beta cells: EMC virus, Mengovirus, and the Coxsackie viruses. When susceptible cells are infected with picornaviruses, the cells are destroyed because of the inhibition of RNA and protein synthesis of the infected cells.

On the contrary, there is also some evidence that viruses such as Lymphocytic choriomeningitis virus (LCMV) (Oldstone, 1988; Dyrb erg et al., 1988) and Mouse hepatitis virus (MHV) (Wilberz et al., 1991) can protect against the development of autoimmune T1D in two spontaneously diabetic animals,
the BioBreeding (BB) rat and the NOD mouse. Furthermore, in view of the disease's globally rising incidence, it is hypothesized that improved hygiene standards may reduce the immune system's ability to appropriately respond to viral infections. The hygiene hypothesis proposes that increased hygiene may cause changes in the composition of gut bacterial flora (Wen et al., 2008), influencing the maturation of the immune system, facilitating imbalance and thereby autoimmune reactions in genetically predisposed individuals (Ludvigsson et al., 2006). In some studies, the frequency of childhood infections correlated inversely with the incidence of T1D (Gibbon et al., 1997; Pundziute et al., 2000).

Arguments in favour of and against viral infections as major aetiological factors in T1D will now be discussed in conjunction with potential pathological scenarios.

1.1.1 Arguments in Favour of Viral Involvement in the Pathogenesis of T1D

What could be the pathological mechanisms that link viral infection to the onset of islet autoimmunity and eventually development of T1D?

One possibility that was put forward by the work of Foulis and co-workers (1987) is that a beta cell-specific viral infection could have the ability to persist and initiate islet inflammation. It was demonstrated that recently diagnosed T1D patients harboured pancreatic islets that expressed aberrantly major histocompatibility complex (MHC) class I and interferon (IFN)-α. Both molecules are up-regulated typically in response to viral infection and could be envisioned to cause recognition and killing of beta cells by infiltrating CD8 T cells. Some reports have indeed documented enterovirus infection specifically within pancreatic islets, and there seems to be a connection with an atypical “fulminant” subtype of T1D (Dotta et al., 2007; Richardson et al., 2009; Tanaka et al., 2009). Nevertheless, these results are in need of further confirmation using complementary detection techniques in order to gauge the precise frequency of beta cell-specific viral infection in T1D versus controls.

The concept of “molecular mimicry” suggests that viruses expressing epitopes resembling certain beta cell structures have the potential to induce cross-reactive immune responses (Coppieters & von Herrath, 2010). Proof of concept was offered with the design of rat insulin promoter-lymphocytic choriomeningitis virus glycoprotein (RIP-LCMV.GP) transgenic mice, which develop diabetes after infection with LCMV (Ohashi et al., 1991; Oldstone et al., 1991). Some potential cross-reactivity has been documented in the past between Coxsackie virus constituents and glutamic acid decarboxylase (GAD) (Kaufman et al., 1992; Atkinson et al., 1994), a major autoantigen in T1D, but this correlation has since been challenged by others (Richter et al., 1994; Horwitz et al., 1998; Schloot et al., 2001). An alternative scenario was proposed based on results in the RIP-LCMV model showing that sequential viral mimicry events can accelerate disease onset (Christen et al., 2004), but such hypotheses are difficult to test in a patient setting.

In contrast, “bystander activation” explains the recruitment and activation of autoaggressive cells to the islet milieu as a consequence of localized viral infection. Virus could lead to activation and maturation of antigen-presenting cells (APCs), which would then shuttle antigen to the pancreatic draining lymph nodes resulting in priming of autoaggressive T cells (von Herrath et al., 2003). The theory was strengthened by the finding that Coxsackie virus infection acts primarily by enhancing the release of islet antigens which, in turn, stimulate resting autoreactive T cells (Horwitz et al., 1998). Bystander activation, caused merely by cytokine released from inflammatory cells and infected cells, is unlikely to be enough to break tolerance (von Herrath et al., 1995; Holz et al., 2001) and by itself give rise to diabetes induction, as studies show that activation of APCs in the pancreas is required for T1D initiation in RIP-LCMV mice (von Herrath et al., 1997; Garza et al., 2000). The observation that enteroviruses are found predom-
inantly around clinical diagnosis may support indirectly the idea that viral infection serves only as a non-specific, one-time trigger to allow pre-existing autoreactive T cells to reach their targets. Studies in the NOD mouse also revealed that a critical mass of autoimmunity is required for Coxsackie virus infections to be diabetogenic (Serreze et al., 2000; Drescher et al., 2004). One attractive mechanism would be that pancreotrophic viruses can precondition the local vasculature to allow entry of effector T cells.

The “fertile-field hypothesis” was conceived to explain how multiple microbial agents could culminate in potentially a single autoimmune disorder. Applied to T1D, the idea is that a viral infection with the right timing may give rise to a transient period, during which the pancreas becomes a fertile field for the development of autoimmune cells. Through induction of beta cell stress and activation of antigen drainage, self-epitopes are then released and presented to self-reactive T cells. In this context, it was found that the contribution of apoptosis-related epitopes during spontaneous development in the NOD mouse, appears to be limited, but this pathway could become enhanced after viral infection (Coppieters et al., 2011). The observation that diabetes acceleration in NOD mice by Coxsackie virus requires a critical level of inflammation contradicts this hypothesis, and indicates that insulitis may, in fact, serve as the “fertilizer” for viruses to inflict any meaningful damage (Serreze et al., 2000; Drescher et al., 2004).

“Genetic predisposition” is obviously a major factor in T1D development. Could it be that individuals with susceptibility genes for T1D possess a greater risk of productive infection or an inability to accurately respond to, e.g. enteroviral infections? Genetic studies indeed suggest that mutations in IFN-response genes might lay at the basis of an exaggerated response to viral infection in type 1 diabetes patients. It should therefore be considered that the observed co-occurrence of enteroviruses and T1D reflects the host’s inability to deal appropriately with a common, normally harmless infection (Jaïdan et al., 2012; Hober et al., 2012).

Finally, it is relevant to mention the aggressive T1D subtype known as “fulminant” T1D. Viral infection is associated primarily with non-autoimmune subtypes of T1D. It is reported predominantly in the Japanese population and is characterized by the absence of autoantibodies, acute onset - often with ketoacidosis - and the almost complete destruction of beta cells at diagnosis. Patients with fulminant T1D often show symptoms of Enterovirus infection prior to onset (Hanafusa et al., 2007), and histological data demonstrate that a significant fraction of pancreas contain enteroviral particles (Tanaka et al., 2009). The apparently strong correlation between enteroviruses and this unconventional, non-autoimmune disease phenotype could mean that at least some less-characterized donors may have been affected by this disease subtype (Richardson et al., 2009).

1.1.2 Arguments Opposed to a Viral Involvement in the Pathogenesis of T1D

“Viral” or “inflammatory” signature? Up-regulation of MHC class I as well as type 1 IFN and IFN-inducible chemokines such as CXCL10 has been observed in pancreas from T1D patients. All these markers are expressed typically in response to viral infection, but also as a consequence of generalized local inflammation. In mouse models, Seewald et al. (2000) demonstrated persistent up-regulation of MHC class I long after viral clearance in diabetic RAT-LCMV.GP transgenic mice. This raises the question of whether MHC class I hyperexpression may be a mere consequence of ongoing inflammation rather than a result of ongoing infection. Can virus persist at all in the pancreas? Although shown only in cardiac tissue to date, it is not known whether a similar persistence can occur in other tissues, although there is no reason at this point to doubt that it could. The question devolves to how long might an HEV persist in any given tissue. It was found MHC class I hyperexpression but no evidence of viral infection
in any of the long-standing T1D donor pancreas, thus suggesting that up-regulation is not caused by any known virus (Kim et al., 2005; Chapman et al., 2008).

Another opinion is that viral agents may represent a minor environmental component in T1D. Throughout history, many inconsistencies have accumulated in the literature with regard to studies linking detection of viral RNA or protein in blood, stool or pancreatic tissue to T1D onset. A recent meta-study by Yeung et al. (2011) that included measurements of Enterovirus RNA or viral capsid protein in blood, stool or tissue of patients with pre-diabetes and diabetes found a significant correlation. An earlier meta-study, in contrast, claimed that no convincing evidence existed for an association between Coxsackie B virus serology and T1D from the 26 examined studies that were included (Green et al., 2004). These discrepancies could be explained by the involvement of several viral strains, many of which are still undiscovered, all of which may affect certain populations differently. Further, it is possible that not a single event, but rather a series of infections is required and that transient infection stages escape detection in cross-sectional studies. Importantly, detection methods are far from standardized, and sensitivity thresholds can be expected to vary wildly. The option should be considered that viral agents represent only a small percentage of the environmental component in T1D and that significance is achieved only within certain susceptible populations. Finland, with its staggering T1D incidence, might be such a region where enteroviral strains contribute more aggressively compared to other countries. Moreover, viral infections could be an epiphenomenon. In a study by Richardson et al. (2009) the observation that 40% of type 2 diabetics showed that the presence of virus in their pancreatic islets may indicate that viral infection is an epiphenomenon to conditions of general beta cell stress. The true infection frequency in T1D should therefore be considered vis-à-vis with other forms of diabetes in order to exclude any secondary effects.

1.1.3 Retrovirus and Diabetes

As most mammalian species contain endogenous retroviruses as part of their DNA, the expression of endogenous retroviruses by beta cells could be associated with insulitis and T1D in NOD mice (Suenaga & Yoon, 1988; Gaskins et al., 1992; Nakagawa et al., 1992). In NOD mice, the islet cells express various retroviral messenger RNAs (mRNAs) encoded by the gag, pol and env genes, and the beta cells in particular express the group-specific antigen p73 of the A-type retrovirus (Pak et al., 1995). In addition, the presence of both A-type and C-type retroviral particles was found in the pancreatic beta cells of NOD mice and was considered to be associated with the development of autoimmune T1D in these animals (Fukino-Kurihara et al., 1985). It is not certain how retroviruses may be involved in the pathogenesis of autoimmune T1D in NOD mice. The presentation of a retroviral antigen on the beta cells by antigen-presenting cells, such as macrophages and dendritic cells may be the initial step in the autoimmune destruction of beta cells. An immune response to a specific antigen on a target cell involves the activation of CD4+ T cells by antigens presented on the surface of a macrophage or other antigen-presenting cells. Studies support this possibility, as elimination of macrophages resulted in the prevention of beta cell-specific autoimmune processes in NOD mice (Lee et al., 1988; Charlton et al., 1988; Jun et al., 1999; Jun & Yoon, 2003). Another possible mechanism whereby retroviruses could be involved in the initiation of autoimmune T1D in NOD mice is the alteration of the expression of cellular genes by the retroviral genomes in the beta cells, possibly resulting in a beta cell-specific altered antigen(s). An altered antigen might be recognized as foreign by immunocytes, leading to beta cell-specific autoimmunity. Besides, it is possible that cellular proteins taken up in the retroviral envelope may elicit an autoimmune response or that IFN-γ-induced expression of HLA-II may trigger autoimmunity through CD4+ lymphocytes.
In addition to animal models such as the NOD mouse, endogenous retroviruses have also been implicated in human T1D. Anti-insulin autoantibodies from T1D patients and their non-diabetic, first-degree relatives have been found to cross-react with the retroviral p73 antigen in up to 75% of cases, whereas only 3% of non-diabetic unrelated controls had p73-binding antibodies, confirming that anti-insulin autoantibody-positive sera contain antibodies that recognize both insulin and p73 (Hao et al., 1993). In other autoimmune diseases, nucleotide sequence homologies are being discovered between human retroviruses and self-antigens, in particular between ribonucleoproteins and the p30 C-type retroviral gag-gene product (Query & Keene, 1987; Nyman et al., 1990; Brookes et al., 1992). Moreover, electron microscope studies demonstrated Retrovirus-like particles in the cytoplasm of beta cells of T1D patients who died shortly after the onset of diabetes, and in none of the non-diabetic controls. All diabetic pancreases showed islet destruction with insulinitis. Retroviral antigens released from beta cells during beta cell turnover might be processed by antigen-presenting cells such as macrophages, dendritic cells or B cells and presented to T-helper cells (CD4+) in association with HLA class II antigens. The activated CD4+ T cells secrete interleukin (IL)-2, which amplifies retroviral antigen-specific CD8+ cytotoxic T cells. These cells could recognize retroviral antigens expressed on beta cells in conjunction with MHC class I antigen, resulting in CD8+ cytotoxic T cell-mediated beta cell destruction. A novel human endogenous retroviral gene, designated IDDMK$_{1,2}$ 22, thought to belong to the mouse mammary tumor virus-related family of human endogenous retroviruses (HERV)-K, was reported to be expressed in the plasma of recent-onset T1D patients but not in nondiabetic control subjects (Conrad et al., 1997). However, careful studies have shown that a sequence identical to that of IDDMK$_{1,2}$ 22 was not present in either the plasma or peripheral lymphocytes from either diabetic or control subjects (Kim et al., 1999). Instead, a related human endogenous retrovirus with 90% to 93% sequence homology with IDDMK$_{1,2}$ 22 was present equally in both diabetic and non-diabetic subjects, indicating that this identified human endogenous Retrovirus is unlikely to be associated with the development of autoimmune T1D in humans (Lower et al., 1998). Even though it appears that the endogenous retroviral gene homologous with IDDMK$_{1,2}$ 22 is not associated with T1D, it does not necessarily exclude the involvement of other human retroviruses or endogenous Retrovirus genes in the pathogenesis of autoimmune diabetes. An interesting report showed that the expression of the defective retroviral gene, the HERV-K18 provirus encoding super antigen, is induced by IFN-α and subsequently stimulates Vβ7 T cells, which was correlated with the onset of T1D. Whether the HERV-K18 provirus is truly involved in the development of autoimmune diabetes remains to be determined (Stauffer et al., 2001).

1.1.4 Reovirus and Diabetes

Reovirus is a double-stranded RNA virus that is believed to cause mild infections of the upper respiratory and gastrointestinal tract of humans.

It has also been associated with T1D in animals; however its mode of action is not known. Mice infected with beta cell-passaged Reovirus type 3 showed abnormal glucose tolerance tests within 10 days after infection, but glucose tolerance returned to normal after three weeks (Onodera et al., 1978). Specific viral antigens were present in some beta cells as well as in acinar cells of these animals, and viral particles were detected by electron microscopy in the cytoplasm of some beta cells, suggesting that the diabetic symptoms were caused by direct infection of the beta cells. Other evidence suggests that Reovirus might cause transient diabetes through an immune reaction. Mice infected with beta cell-passaged Reovirus type 1 developed transient diabetes, and their sera contained auto-antibodies that reacted with cytoplasmic antigens from the islets of Langerhans, the anterior pituitary, and the gastric mucosa of uninfec-
ed mice (Onodera et al., 1981). An autoimmune mechanism might be involved in the disease because of the administration of immunosuppressive drugs to Reovirus-infected SJL and NFS mice reduced or prevented the development of Reovirus-induced diabetes and mortality. Moreover, other studies suggest that a Th1 response induced by the increased expression of IL-12 may be responsible for the development of diabetes in newborn DBA/1 mice infected with Reovirus (Hayashi et al., 2001). Human beta cells are also susceptible to Reovirus type 3 infection in vitro (Yoon et al., 1981); however there is little evidence for the involvement of Reovirus in the pathogenesis of human T1D.

1.1.5 Kilham Rat Virus and Diabetes

Kilham rat virus (KRV) belongs to the Paroviridae family and it was originally isolated from a rat sarcoma, it has been found to cause a fatal neonatal disease, physical deformities and mental retardation in newborn rats.

KRV has been shown to induce diabetes by provoking autoimmune responses against the beta cells, rather than by direct beta cell infection in diabetes-resistant BB (DR-BB) rats (Guberski et al., 1991; Brown et al., 1993). Diabetes-prone (DP)-BB rats, like NOD mice, spontaneously develop a diabetic syndrome that resembles human T1D in many respects (Marliss, 1983). DP-BB rats are lymphopenic and 80% to 100% of the animals become diabetic at about 120 days of age. DR-BB rats are derived from DP-BB rats, but do not normally develop diabetes. When DR-BB rats were infected with KRV at three weeks of age, about 30% of these animals developed autoimmune diabetes within two to four weeks after infection and a further 30% showed insulitis without diabetes (Guberski et al., 1991). Because of the inactivation of macrophages with liposomal dichloromethylene diphosphonate (lip-Cl₂MDP), which selectively destroys macrophages by apoptosis, results in the near complete prevention of insulitis and diabetes in KRV-infected DR-BB rats, macrophages and macrophage-derived cytokines play a critical role in the cascade of events leading to the destruction of pancreatic beta cells, culminating in the development of autoimmune diabetes in KRV-infected DR-BB rats (Chung et al., 1997). Experimental data suggests that KRV infection leads to the activation of silent autoreactive T cells that are specific for beta cells in DR-BB rats (Ellerman et al., 1996). However, the precise mechanism by which KRV induces autoimmune T1D without the infection of beta cells is poorly understood. In addition, it was unclear how KRV-specific autoreactive T cells destroy pancreatic beta cells without direct infection of the cells by KRV. It was hypothesized that KRV antigen-specific T cells generated by KRV peptides might cross-react with pancreatic beta cells and attack them, resulting in the development of insulitis and, subsequently, diabetes. Chung and colleagues (2000) indicated that molecular mimicry between KRV peptides and beta cell-specific autoantigens in DR-BB rats is unlikely to be a mechanism by which KRV induces beta cell-specific autoimmune diabetes.

Another possibility is that KRV infection of DR-BB rats might disturb the finely tuned immune balance and activate autoreactive T cells that are cytotoxic to beta cells, resulting in T cell-mediated autoimmune diabetes similar to that seen in DP-BB rats. To test this hypothesis, the CD4⁺ and CD8⁺ T-cell populations were examined in the splenocytes of DR-BB rats after KRV infection. The percentage of CD8⁺ T cells increased considerably, whereas the percentage of CD4⁺ T cells decreased, although the absolute number of both CD4⁺ and CD8⁺ T cells was increased during KRV infection. In addition, CD8⁺ T cells preferentially proliferated as compared with CD4⁺ T cells in KRV-infected DR-BB rats (Chung et al., 2000). Moreover treatment of KRV-infected DR-BB rats with OX-8 monoclonal antibody significantly decreased the incidence of diabetes, indicating that CD8⁺ T cells are clearly involved in the destruction of beta cells. It has been reported that the treatment of DP-BB rats with anti-NK cell antibody failed to
prevent diabetes, while OX-8 monoclonal antibody treatment successfully prevented diabetes (Ellerman et al., 1993). Therefore, it is more likely that CD8\(^+\) T cells may play a major role in KRV-induced diabetes, although the possibility of the involvement of NK cells cannot be absolutely excluded, because OX-8 monoclonal antibody also depletes NK cells.

It has been suggested that the dominance of Th1 cells over Th2 cells is associated with the development of autoimmune T1D, whereas the dominance of Th2 cells over Th1 cells is associated with the prevention of T1D (Rabinovitch, 1994; Liblau et al., 1995; Delovitch & Singh, 1997). KRV infection in DR-BB rats increased the expression of Th1-type cytokines in the splenocytes and pancreatic infiltrates (Chung et al., 1997); therefore, it is possible that the proportions of Th1 and Th2 cells are altered during KRV infection in DR-BB rats. Subsequent experiments showed that the number of Th2-like CD45RC\(^-\) CD4\(^+\) T cells was significantly decreased and the number of Th1-like CD45RC\(^+\) CD4\(^+\) T cells significantly increased in the splenocytes of KRV-infected DR-BB rats as compared with PBS-treated controls. In addition, Th1-like CD45RC\(^+\) CD4\(^+\) and CD8\(^+\) T cells isolated from DR-BB rats after infection with KRV could induce diabetes in 88% of recipient DP-BB rats when both CD45RC\(^+\) CD4\(^+\) and CD8\(^+\) T cells were transferred (Chung et al., 2000). This result indicates that CD45RC\(^+\) CD4\(^+\) and CD8\(^+\) T cells are major effector T cells that can induce autoimmune diabetes. The incidence of diabetes in DP-BB rats that received either CD45RC\(^+\) CD4\(^+\) or CD8\(^+\) T cells alone was, however, significantly lower as compared with that in rats that received a combination of CD45RC\(^+\) CD4\(^+\) and CD8\(^+\) T cells. These results indicate that Th1-like CD4\(^+\) and CD8\(^+\) T cells from KRV-infected rats work synergistically to destroy pancreatic beta cells, as proposed previously (Chung et al., 1997). In contrast, none of the recipients of both CD45RC\(^-\) CD4\(^+\) and CD8\(^+\) T cells developed diabetes, indicating that CD45RC\(^-\) CD4\(^+\) T cells play a role as regulatory T cells. Therefore, infectious KRV, rather than KRV proteins expressed in rVV\(s\), is absolutely required to disturb or breakdown the finely tuned immune balance, resulting in the upregulation of preexisting beta cell-specific autoreactive T cells that can destroy beta cells.

1.1.6 **Bovine Viral Diarrhea Virus and Diabetes**

Bovine viral diarrhea virus (BVDV) belongs to Pestivirus genus of the Flaviviridae family and is widespread in livestock such as cattle. BVDV has been reported to be associated with T1D in cattle, however not all animals with BVDV infection develop diabetes (Tajima et al., 1992). This may be attributable to the existence of different variants of the virus or to genetic differences among the hosts. In a more recent study, BVDV infected cattle with T1D showed the presence of BVDV genes in the pancreas, however, not in the islet cells. Many of these cattle also had islet cell autoantibodies, suggesting that T1D associated with BVDV is not a direct effect of BVDV on islet cells (Tajima et al., 1999).

1.1.7 **Mumps Virus and Diabetes**

The **Mumps virus** is an enveloped single-stranded virus belonging to the Paramyxoviridae family. Mumps virus was one of the first viruses implicated in the development of human T1D; several cases were reported in which mumps infection appeared to precede the onset of T1D (Gamble, 1980).

It has been hypothesized that infection with Mumps virus may induce autoimmunity, as some children appear to develop islet cell autoantibodies during parotiditis (Helmke et al., 1980), however the mechanisms by which this might occur are unknown. In vitro studies have shown that human beta cells could be infected with Mumps virus (Prince et al., 1978), that mumps infection of a human insulinoma cell line induced the release of IL-1 and IL-6 and upregulated the expression of HLA class I and II antigens (Cavallo et al., 1992), and that pancreatic beta cells infected had increased expression of only HLA
class I molecules (Parkkonen et al., 1992). In addition, Mumps virus has been shown to be capable of replicating in the exocrine pancreas (Vuorinen et al., 1992). On the basis of these studies, it may be suggested that cytokines released by Mumps virus-infected cells and increased expression of HLA molecules by infected beta cells may lead to an immune response against the beta cells or may increase preexisting autoimmune processes directed against beta cells. Several studies have explored the impact of mumps vaccinations on either increasing or decreasing the incidence of T1D. One investigation concluded that the elimination of natural mumps infections by vaccination may have been responsible for the decreased risk of developing T1D (Hyöty et al., 1993). Other studies concluded that there is no association with childhood mumps vaccinations and the development of islet autoimmunity (Hummel et al., 2000) or T1D (De Stefano et al., 2001).

1.1.8 Rubella Virus and Diabetes

Rubella virus is a non-segmented, single-stranded RNA enveloped virus that belongs to the Togaviridae family. It has been implicated in T1D. Patients with congenital rubella syndrome (CRS) had a higher incidence of T1D than the general population, with approximately 10% to 20% developing diabetes between the ages of 5 to 20 years (McEvoy et al., 1986). Islet cell and anti-insulin antibodies were found in 50% to 80% of diabetic patients with CRS, whereas these antibodies were present in about 20% of non diabetic patients with CRS (Ginsberg-Fellner et al., 1984), suggesting an underlying autoimmune disorder. Genetic susceptibility may also be involved (Ginsberg-Fellner et al., 1986).

Rubella virus appears to be able to directly infect beta cells, as shown by in vivo and in vitro studies. Neonatal golden Syrian hamsters infected with beta cell-passaged Rubella virus developed hyperglycemia and hypoinsulinemia between 7 and 10 days of age, and their beta cells were positive for Rubella virus antigen. An autoimmune process may be involved, as 40% of infected animals had cytoplasmic islet cell antibodies and 34.5% had insulitis (Rayfield et al., 1986). Human islets are also susceptible to direct Rubella virus infection under culture conditions. Human fetal islets exposed to Rubella virus contained rubella viral antigens in both beta and non beta cells and had lowered levels of insulin production (Numazaki et al., 1989), although without any demonstrable cytopathology (Numazaki et al., 1990). It is possible that the virus may insert, expose, or alter antigens in the plasma membrane of the infected host as it buds through the cell membrane. Rubella viral antigens on beta cells or Rubella virus-altered antigens on the surface of beta cells may be perceived as foreign by the host's immune system, leading to beta cell-specific autoimmunity.

Alternatively, in vitro studies suggest that Rubella virus may induce autoimmune T1D by molecular mimicry. When a panel of monoclonal antibodies that recognize rubella virus capsid and envelope glycoproteins were tested for reactivity with islet cell antigens, one monoclonal antibody that recognized a domain within the Rubella virus capsid protein was found to react with extracts from rat and human islets, as well as with extracts from a rat insulinoma line (Karounos et al., 1993). Further testing showed that the shared epitope was on a 52-kD protein. In addition, it was reported that T cells of diabetic patients recognize cross-reactive protein determinants from Rubella virus and glutamic acid decarboxylase (GAD) 65 and 67 (Ou et al., 2000), which is considered to be an important β-cell autoantigen in the pathogenesis of T1D. This suggests that Rubella virus exposure may lead to the generation of viral antigen-specific cytotoxic T cells that also recognize beta cell-specific antigen(s) in susceptible individuals.
1.1.9 Cytomegalovirus and Diabetes

Cytomegalovirus (CMV) belongs to the Herpesviridae family and is a double-stranded DNA enveloped virus. Like Rubella virus, human CMV can be congenital, although the disease may not appear until later in life. CMV infections can also be transmitted perinatally or postnatally through close contact or breast milk, as the immaturity of infant immune systems favors the establishment of persistent viral infections.

CMV has been implicated in T1D by a number of clinical studies. Case reports describe a child with congenital CMV infection (Ward et al., 1979) and a woman with CMV infection (Yasumoto et al., 1992) who both developed T1D, the latter after extensive pancreatitis. In a study of children with fatal viral infections, viral cytopathology of the pancreas and characteristic inclusion bodies in the beta cells were found in 20/45 cases of CMV infection (Jensen et al., 1980), indicating that CMV can infect pancreatic beta cells. In another study following 73 infants with congenital CMV infection, one developed T1D, compared to 38/19,483 non infected control subjects, which the investigators believed indicated no statistical correlation between CMV infection and the development of T1D (Ivarsson et al., 1993). Correlations between CMV infection and T1D also have been found by studies using molecular biological methods. A research using both dot and in situ hybridization techniques showed that 20% of T1D patients had cytomegalovirus genomic DNA in their lymphocytes, compared to only 2% of normal controls. Furthermore, 80% of patients who had both anti-CMV antibodies and the CMV genome also had islet cell autoantibodies (Pak et al., 1988). Nicoletti et al. (1990) found that non diabetic siblings of T1D patients had a significant association between high titers of anti-CMV antibodies and islet cell auto-antibodies, but no correlation between anti-CMV antibodies with HLA-DR antigens. These findings suggest that chronic CMV infection may be associated with islet cell autoantibody production, but that other factors may be needed for the development of clinical T1D. It is possible that molecular mimicry may be involved in some cases of CMV-induced diabetes. In this situation, immune responses against similar epitopes shared by antigenic determinants of CMV and islet cell-specific proteins may lead to islet cell-specific autoimmunity. Evidence for this is the finding that human CMV can induce an islet cell antibody that reacts with a 38-kD auto-antigen expressed in human pancreatic islets (Pak et al., 1990). Also, a work showed that a CD4 T-cell clone reactive to GAD65 isolated from a prediabetic Stiffman syndrome patient cross-reacted with a peptide of human CMV major DNA binding protein, suggesting that human CMV may be involved in the induction of autoimmunity by molecular mimicry of the beta cell autoantigen, GAD65 (Hiemstra et al., 2001). Further studies are needed to determine whether CMV is actually involved in the development of T1D in man and/or animals because the evidence of the association of CMV with T1D remains circumstantial.

1.1.10 Epstein-Barr Virus and Diabetes

Epstein-Barr virus (EBV) is a double-stranded DNA enveloped virus that belongs to the Herpesviridae family. EBV has been implicated in the etiology of autoimmune diseases (Hyöty et al., 1991; Parkkonen et al., 1994). A temporal link between EBV infection and the onset of T1D has been reported in a rare number of cases, including one in which the child also had concurrent Adenovirus and Coxsackie B viral infections (Surcel et al., 1988).

There is some evidence that EBV may be potentially capable of triggering autoimmune T1D by molecular mimicry. An 11 amino acid sequence of the EBV protein, BOLF1, was found to be homologous to residues in the Asp-57 region of the HLA-DQw8 beta chain peptide, although sera from the diabetic patients tested by Sairenji et al. (1991) did not bind to DQw8 beta. It was also found that a pen-
tapeptide sequence in the Asp-57 region of the HLA-DQβ chain is successively repeated six times in the EBV-BERF4-encoded epitope (Horn et al., 1988). Two patients who produced antibodies against this epitope during acute EBV infection soon developed T1D, while five individuals also acutely infected but not producing antibodies against this epitope did not develop T1D (Parkkonen et al., 1994).

### 1.1.11 Encephalomyocarditis Virus and Diabetes

There is unequivocal evidence that diabetogenic variants of Encephalomyocarditis virus (EMCV), a member of Picornaviridae family, can induce diabetes in animals (Yoon J et al., 1980), although there is little evidence that this occurs in humans. Infection of genetically susceptible mice with the M variant of EMCV virus resulted in the selective destruction of pancreatic beta cells (Stefan et al., 1978). Further searches found that beta cell destruction in EMCV-infected mice is dependent on the genetic makeup of the virus and the genetic background of the host (Kang et al., 1993; Jun & Yoon, 2001). Two different animal models for EMCV-induced diabetes are supposed. The first model involves animals infected with a high dose (10^5 PFU/mouse) of EMCV-induced diabetes, in which replication of EMCV within the beta cells plays a major role, whereas recruitment of macrophages plays a minor role in beta cell destruction. In contrast, the second model involves animals infected with a low dose (<10^5 PFU/mouse) of EMCV-induced diabetes, in which activated macrophages that are recruited to the beta cells play a major role, whereas replication of the virus within the beta cells plays a minor role in beta cell destruction. The development of diabetes in these infected mice is mainly due to the replication of the EMCV within the beta cells, rather than to the involvement of humoral and/or cell-mediated immune responses. Although T cells do not appear to be involved, it is possible that macrophages might contribute to the destruction of pancreatic beta cells in mice infected with a high dose of EMCV. Macrophages predominate in the pancreatic islets during the early stages of EMC viral infection (Baek & Yoon, 1990), and it is possible that activated macrophages might migrate to EMCV-infected beta cells as scavengers and secrete cytotoxic cytokines such as IL-1, TNF-α and IFN-γ and nitric oxide. In this way, the active replication of EMCV-induced diabetes within the beta cells and the production of cytokines and oxygen free radicals from activated macrophages could act synergistically to destroy beta cells, leading to the development of diabetes.

As natural viral infections in animals and man generally involve exposure to relatively low numbers of virus, and exposure to the high viral titers would be unlikely in nature, another animal model was established to study the immune mechanisms involved in the destruction of beta cells, in which mice were infected with a low dose (100 PFU/mouse) of EMCV-induced diabetes. In mice infected with a low dose of EMCV-induced diabetes, macrophages play a central role in the destruction of pancreatic beta cells, as activation of macrophages prior to viral infection results in a statistically significant increase in the incidence of diabetes, and inactivation of macrophages prior to viral infection almost completely prevents EMCV-induced diabetes (Baek & Yoon, 1991). Additional studies showed that the selective EMCV-induced diabetes viral infection of pancreatic beta cells results in an initial recruitment of macrophages into the islets, followed by infiltration by other immunocytes including T cells, NK cells and B cells (Baek & Yoon, 1990). Further study revealed that EMCV-induced diabetes infects macrophages and activates them, but does not replicate within the macrophages (Hirasawa et al., 1999). The expression of IL-1β, TNF-α and inducible nitric oxide synthase (iNOS) was selectively detected in the pancreatic islets of mice infected with a low dose of EMCV-induced diabetes. In addition, treatment of EMCV infected mice with antibody against IL-1β or TNF-α or with the iNOS inhibitor, aminoguanidine, exhibited a significant decrease in the incidence of diabetes (Hirasawa et al., 1997). These results suggest that macrophage-derived soluble mediators play a critical role in the destruction of pancreatic beta cells resulting in
the development of diabetes in mice infected with a low dose of EMCV-induced diabetes. Further investigations regarding the mechanisms that activate macrophages found that tyrosine kinase signaling pathways are involved in the EMCV-induced activation of macrophages. It was recently found that an Src family kinase, hematopoetic cell kinase (hck), showed a dramatic increase in autophosphorylation and phosphorylation of Sam 68 (a substrate for Src kinase) in EMC-D virus-infected mice. The protein level of hck had a peak at 48h after the infection of EMCV, suggesting that hck is involved in the activation of macrophages in mice infected with EMC-D virus. Treatment of EMC-D virus-infected mice with an Src kinase inhibitor, PP2, resulted in the inhibition of hck activity, a decrease in the production of TNF-α and iNOS in the macrophages and the subsequent prevention of diabetes (Choi et al., 2001).

1.1.12 Mengovirus and Diabetes

Mengovirus is a member of the Cardiovirus genus of Picornaviridae family, like EMCV, and produces fatal encephalitis in mice. While Mengovirus is antigenically similar to EMCV, it is more neuropathic and lethal and has different tissue tropisms (Morishima et al., 1982). Plaque purification of Mengovirus resulted in the isolation of a clone, Mengovirus-2T, which caused diabetes in strains of mice resistant to EMCV-induced diabetes infection. Pancreas from Mengovirus 2T-infected mice revealed marked beta cell necrosis, severe inflammatory infiltration of the islets, and decreased insulin content, without evidence of autoimmune responses (Yoon et al., 1984). It appears that Mengovirus-2T acts by directly infecting beta cells, and it may bind to a different beta cell receptor than EMCV, thus accounting for the difference in strain susceptibility between the two viruses.

1.1.13 Coxsackie Virus and Diabetes

There is considerable information that T1D may be associated with Enterovirus infection, including infection with Coxsackie viruses and Echoviruses.

There have been many epidemiological studies linking recent-onset T1D with Coxsackie A and B viral infections in humans (Fohlman & Friman, 1993; Yoon et al., 1996). Several studies have reported that patients with recent-onset T1D had significantly higher titers of antibody to Coxsackie virus, especially the B4 serotype, than did non diabetic controls (Friman et al., 1985; Frisk et al., 1992). In addition, it was reported that T-cell responses to Coxsackie B4 nonstructural protein of the virus increased in new-onset T1D patients (Juhela et al., 2000). While these studies support Coxsackie B viral involvement in the development of human T1D, results from epidemiological studies have been inconsistent. Some works have found no evidence of a correlation between the onset of T1D and Coxsackie B viral infections (Pato et al., 1992; Marttila et al., 2001), while other studies have found higher levels of anti-Coxsackie B virus-specific antibodies in non diabetic control subjects than in recent-onset T1D patients (Tuveo et al., 1989). The interpretation is complicated by the fact that there are many variants of Coxsackie B4 virus (Prabhakar et al., 1982), and only a minority is likely to cause diabetes: four Coxsackie B4 variants were tested and only one proved to cause diabetes in mice, while the remaining three variants did not (Yoon et al., 1986). Moreover, the lack of correlation may depend on the genetic makeup of the virus and differences in immune responses among individuals. Many anecdotal reports described the development of T1D in patients with recent or concurrent Coxsackie B viral infections (Nigro et al., 1986; Andreoletti et al., 1997). There are several direct supporting evidences on the association of Coxsackie B viral infection with the onset of T1D (Dotta et al., 2007). In vitro studies have shown that Coxsackie B3 virus and Coxsackie B4 virus can infect and impair human islet beta cells metabolism (Szopa et al., 1986). These works
have shown that the insulin content of infected beta cells decreased rapidly, beginning at 24h after infection, and that the decrease in insulin roughly paralleled the increase in viral titer.

Not all variants of Coxackie B4 virus cause overt diabetes in susceptible animals explaining why T1D appears to be associated with Coxackie B viral infection in infrequent isolated cases. In early studies, mice inoculated with Coxackie B4 virus did not develop diabetes; however repeated passaging of Coxackie B4 in murine-enriched pancreatic beta cell cultures resulted in the virus acquiring more diabetogenic capacity (Yoon et al., 1978). Mice infected with this virus showed lymphocytic infiltration of the islets and beta cell destruction, which lead to hypoinsulinemia and hyperglycemia. An inverse correlation was observed between the reduction in immunoreactive insulin and the elevation in blood glucose levels (Yoon et al., 1978; Toniolo et al., 1982). In the majority of animals, hyperglycemia is transient. It is possible that sufficient beta cells remain intact after some Coxackie B4 viral infections so that proliferation and/or hypertrophy of these cells results in metabolic compensation. During the acute phase of Coxackie B4 viral infection, viral antigens have been found in the islets of Langerhans. Genetic susceptibility of the host plays an important role in Coxackie B-induced diabetes in animals, as is the case with EMCV. Genetic studies showed that the 'db' diabetic mutation on chromosome 4 exerted the most effect on susceptibility and host response to Coxackie B4 virus and was associated with an impaired humoral response to Coxackie B4 viral infection as infected mice did not develop an adequate level of anti-Coxackie B4 IgM and IgG antibodies. The animals were also found to be deficient in absolute and relative numbers of splenic lymphocyte subsets. It has also been reported that Coxackie B4 viral infection alters thymic, splenic, and peripheral lymphocyte repertoire before the onset of hyperglycemia in mice (Chatterjee et al., 1992).

Coxackie B viruses may induce diabetes through several mechanisms. Coxackie B viruses affect glucose homeostasis. The virus has cytolytic activity, and in animals, may directly destroy enough of the beta cell mass to cause T1D (Toniolo et al., 1982). Autoimmune mechanisms may also be involved, perhaps because the immune response rose against the virus cross-reacts with specific beta cell antigens. P2-C, a non-capsid protein of Coxackie B4, has sequence homology with GAD, which is expressed by beta cells and is a putative autoantigen (Kaufman et al., 1992). Moreover, infection with the virus increases expression of GAD by beta cells (Hou et al., 1993). Antibodies have been detected in T1D patients that react with both P2-C and GAD (Hou et al., 1994), suggesting that this ‘molecular mimicry’ could underlie the autoimmune damage to the beta cells. However, this hypothesis is not supported by studies that characterized antibodies produced by lymphocytes isolated from a newly diagnosed T1D patient. Four of six antibodies studied recognized and bound to the region of GAD65 that is homologous to P2-C, but none cross-reacted with P2-C itself or with any other Coxackie B4 viral proteins. The lack of cross-reactivity between these two proteins may be due to differences in secondary or tertiary structure (Richter et al., 1994). On the other hand, the capacity of murine T lymphocytes to cross-react with P2-C and GAD is associated with a diabetes susceptibility allele; cross-reactive T-cell recognition of GAD65 may therefore contribute to the initiation or amplification of autoimmune responses against the beta cell, and perhaps to the association of T1D with certain HLA alleles (Tian et al., 1994). Coxackie B virus may also induce diabetes by bystander activation of autoreactive T cells against islet antigens. Mice with susceptible MHC alleles had no viral acceleration of diabetes, but mice with a T-cell receptor transgene specific for a different islet autoantigen rapidly developed diabetes. This suggests that Coxackie B virus-induced diabetes by a direct local infection leading to inflammation, tissue damage and the release of sequestered islet antigens resulting in the restimulation of resting autoreactive T cells (Horwitz et al., 1998). A further possibility is that a defective Coxackie B virus, lacking the usual high lytic activity, could cause persis-
tent infection of beta cells, resulting in autoimmune beta cell destruction (Foulis et al., 1990). In addition, it was reported that the level of IFN-α was elevated in the plasma of T1D patients, and this was associated with Coxsackie B virus infection (Chehadeh et al., 2000). The persistent infection of beta cells with Coxsackie B viruses may result in expression of IFN-α, which in turn could induce HLA class I antigen hyperexpression and expression of chemokines that recruit and activate macrophages and T cells. These activated immunocytes could kill beta cells, resulting in T1D. Finally, Coxsackie viral infections may be involved in the pathogenesis of T1D by acting as the terminal insult in individuals who have already lost substantial beta cell mass through ongoing autoimmune damage. Destruction of a critical number of residual cells would result in the clinical onset of T1D.

1.1.14 Hepatitis C virus and Diabetes

While patients with liver disease are known to have a higher prevalence of glucose intolerance, studies suggest that infection with Hepatitis C virus (HCV), a member of Flaviviridae family, may be an additional risk factor for the development of diabetes mellitus (Mason et al., 1999).

Humans with various forms of liver disease can be predisposed to impaired glucose tolerance because of corticosteroid and hydrochlorothiazide therapy or hemochromatosis (Muting et al., 1969; Petrides, 1994; Niedereau et al., 1985). In addition, glucose intolerance is observed more often in patients with HCV infection compared with controls with liver disease (Allison et al., 1994; Ozyilkan & Arslan, 1996), and the frequency of HCV infection in European populations with type II diabetes has been reported to be higher than expected compared with the general population (Ozyilkan et al., 1994; Gray et al., 1995; Simo et al., 1996). HCV infection cannot be considered to be a cause of diabetes without establishing a temporal relationship for the development of disorder.

1.2 Bacterial Infections

The bacterial composition of the intestine has long been acknowledged as an important variable affecting T1D development. Direct evidence exists in rodents, for example, feeding probiotic bacterial strains, usually lactic acid bacteria, to non-obese diabetic (NOD) mice or bio breeding diabetes-prone (BB-DP) rats can delay or prevent diabetes (Matsuzaky et al., 1997; Calcinaro et al., 2005; Yadav et al., 2007). Feeding antibiotics to NOD mice or BB-DP rats can also increase survival in these models (Brugman et al., 2006; Schwartz et al., 2007). In addition, pathogen-free NOD mice lacking an adaptor protein for multiple toll-like receptors known to bind to bacterial ligands fail to develop diabetes (Wen et al., 2008). Perhaps autoimmunity ensues whenever the intricate microbial balance in the intestine is disturbed. Additionally, the intestinal wall does not seem to have the same capacity to form a coherent barrier separating luminal bacteria and the immune system in T1D models and patients versus controls. This so-called-leaky gut phenotype is thought to enhance the exposure of bacterial antigens to the immune system. In the intestine of T1D patients, subclinical immune activation (Westerholm et al., 2003) and evidence for an impaired regulatory T cells (T reg) subset (Tiittanen et al., 2008) were found. T reg are a specialized subpopulation of T cells which suppresses activation of the immune system and thereby maintains tolerance to self antigen. Thus, both antibiotics and probiotics may influence T1D development by altering the balance of gut microbiota toward either a tolerogenic or nontolerogenic state, depending on constitution of the intestinal microflora at the time of administration (Vaarala et al., 2008).

Chronic bacterial infections could be contributing to the socioeconomic gradient in chronic diseases. Although chronic infections have been associated with increased levels of inflammatory cytokines and cardiovascular disease, there is limited evidence on how infections affect risk of diabetes. Studies suggest
that *Chlamydia pneumoniae*, and *Helicobacter pylori*, may have an impact on cardiovascular conditions and metabolic syndrome (Georges et al., 2003; Nabipour et al., 2006) potentially mediated by elevations in inflammatory markers such as C-reactive protein (CRP) and interleukin (IL)-6 (Georges et al., 2003). Inflammation and activated innate immunity have also been implicated in the pathogenesis of diabetes through insulin resistance. Elevated levels of inflammatory cytokines may lead to phosphorylation of serine residues on the insulin receptor substrate, which prevents its interaction with insulin receptors, inhibiting insulin action (Wellen & Hotamisligil, 2005).

### 1.2.1 *Helicobacter Pylori* and Diabetes

There are little, equivocal available contradictory data on *Helicobacter pylori* prevalence and its relationship to Type 2 diabetes mellitus (T2D) in the literature (Gasbarrini et al., 1998; Oldensbury et al., 1996; Ko et al., 2001; Gulcelik et al., 2005).

Many authors reported high prevalence of *H. pylori* infection among patients with T2D, suggesting that delayed gastric clearance could be attributed to bacterial colonization or overgrowth in the gastrointestinal tract as a result of autonomic neuropathy (Albaker, 2011). Some works, based on serologic antibody detection, have found a high prevalence of *H. pylori* infection among diabetics as compared to the general population (Gasbarrini et al., 1998; Gulcelik et al., 2005). On the other hand, another study with histopathological demonstration of microorganism, described a minor role of *H. pylori* infection among upper gastrointestinal pathologies (Malecki et al., 1996), while other reports did not detect a relationship between *H. pylori* infection and diabetes mellitus (Woodward et al., 2000; Hia et al., 2001; Ko et al., 2001; Demir et al., 2008; Lutsey et al., 2009). Jeon and colleagues (2012) found a positive association between *H. pylori* infection and the prevalence of T2D in cross-sectional studies, in accord with the results from previous studies carried out by Gasbarrini et al. (1998) and So et al. (2009). They were able to establish the relative timing of seropositivity and development of diabetes, giving more credence to a potential causal relationship. The mechanism by which *H. pylori* infection increases the risk of diabetes remains to be elucidated but may involve inflammation or dyspepsia. Infection with *H. pylori* was found in previous studies to be correlated with elevated levels of CRP (Diomedi et al., 2008), IL-6, and TNF-α (Hamed et al., 2008), which are markers of inflammation implicated in insulin resistance and development of diabetes. Furthermore, the presence of Gram-negative bacteria, such as *H. pylori*, in the gut microbiota leads to increased production of lipopolysaccharide, which also activates innate inflammatory processes (Manco et al., 2010). An alternative hypothesis is that gastroduodenal conditions resulting from *H. pylori* infection could delay gastric emptying (Ojetti et al., 2010), which has been postulated to cause poor glucose control in insulin-dependent children with diabetes. The biological impact of *H. pylori*-associated disorders on glucose metabolism and insulin sensitivity should be further investigated.

### 1.2.1 Does tuberculosis Lead to Diabetes?

There is growing evidence that diabetes mellitus is an important risk factor for tuberculosis and might affect disease presentation and treatment response (Dooley & Chaisson, 2009).

If diabetes can predispose a patient to tuberculosis, can infection with tuberculosis lead to diabetes mellitus? The answer is: “it is possible”. Tuberculosis might induce glucose intolerance and worsen glycogenic control in people with diabetes. Some studies suggest that tuberculosis can even cause diabetes in those not previously known to be diabetic. Many studies have used oral glucose tolerance testing to show that patients with tuberculosis have higher rates of glucose intolerance than community controls. (Nichols, 1957; Zack et al., 1973; Abbras, 1991). Whereas the high incidence of abnormal oral glucose toler-
ance found in tuberculosis patients is of concern, it is unclear whether glucose intolerance or diabetes mellitus was truly incident, or whether prevalent diabetes mellitus was being newly diagnosed in patients receiving expanded medical services related to tuberculosis treatment. Also, the implications of these findings depend on whether diabetes mellitus persists in these patients, and whether its presence is substantially more common with tuberculosis than with other infectious diseases.

In a study carried out in Nigeria, tuberculosis patients with impaired glucose tolerance had normal tests after 3 months of tuberculosis treatment (Oluboyo & Erasmus, 1990). In Turkey, oral glucose tolerance tests were given to 58 patients with active tuberculosis and 23 patients with community-acquired pneumonia (Basoglu et al., 1999). Of those with tuberculosis, 10% were glucose intolerant and 9% had diabetes; of patients with community-acquired pneumonia, none had glucose intolerance and 17% were diabetic. All patients had normal tests 3 months and 2 years after the start of treatment. The latter two studies suggest that infection causes reversible glucose intolerance and that this effect is not specific to tuberculosis. In Indonesia, 13% (60 of 454) of patients with tuberculosis had diabetes, compared with 31% (18 of 556) of age-matched and sex-matched controls from the same residential unit; for 60% of these patients, diabetes was a new diagnosis. Whereas impairment of glucose metabolism probably preceded tuberculosis in these patients rather than the reverse, these data underscore the importance of screening tuberculosis patients for diabetes.

Another recently discovered bacterial risk factor maybe *Mycobacterium avium* subspecies *paratuberculosis* (MAP).

## 2 Mycobacterium Avium Subsp. Paratuberculosis: Zoonotic Potential

Recently, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) has been postulated as the infectious agent that triggers human Type 1 (insulin-dependent) diabetes mellitus (T1D) (Dow, 2006; Sechi et al., 2008; Rani et al., 2010). Moreover, an association between MAP infection and Crohn’s Disease (CD) human patients has been suggested.

Who is it MAP? MAP is the member of the *Mycobacterium avium* complex and it is the causative pathogen of the chronic, granulomatous degenerative enteritis known as Paratuberculosis or Johne’s disease (JD) that mainly affects domestic and wild ruminants (Chiodini et al., 1984; Buergelt et al., 2000). This bacterial pathogen is also incriminated in the infection of many monogastric animals, including humans (Beard et al., 2001; Hermon-Taylor et al., 2000; Ghadiali et al., 2004). The degree of occurrence of JD is increasing worldwide (Manning & Collins, 2001) with a significant economic impact on the livestock industry (Harris & Barletta, 2001). The disease represents a sanitary and zootechnical problem of remarkable proportions because of its incidence, the lack of a valid therapeutic and preventive strategies to curtail its spread, and the economic losses it incur due to complications from the clinical and subclinical disease (Cocito et al., 1994; Ott et al., 1999; Pavlik et al., 2009). The infection of animals in early life occurs mostly via the faecal-oral route (Sweeney, 1996). Nevertheless, the intrauterine route of transmission has been observed in sheep and in the fetuses of cows with advanced forms of JD (Sweeney, 1996; Lambeth et al., 2004). Granulomatous lesions in the intestine and the involvement of the neighbouring lymph nodes are some hallmark characteristics of MAP infection, and the composition of cell types within the lesions is correlated with stage of infection (Miomotani et al., 1988; Chiodini, 1996). As the disease progresses, the tuberculoid or TH1 response handover to a lepromatous or TH2 response which is characterised by the production of IL-4, IL-5, IL-6 and IL-10 cytokines and an influx of inflammatory cells to
the infection site. The intestinal wall becomes thickened as a result of the intestine inflammation leading to poor nutrient absorption in the affected animals (Williams et al., 1979; Shulaw et al., 1993). The TH2 response is responsible for the activation and sustainability of the humoral immune response characterized by antibody production. Clinical symptoms, such as diarrhea and severe emaciation or wasting, are often observed at this stage of the disease.

The manifestation of the clinical disease which is typically characterized by loss of weight, diarrhea and decreased milk production in dairy herds (Chiodini et al., 1984), and the high shedding of the bacteria in feces and milk is commonly noticeable as from 2 years of age and above, both in clinically and subclinically affected animals (Streeter et al., 1995; Olsen et al., 2002). The affected animals are emaciated and produce less milk despite having a ravenous appetite and are often a-febrile. And as the disease become chronic, death results following severe wasting and diarrhea.

Some studies (Sherman et al., 1990; Singh et al., 2007) have observed that high mortality rates and reduced reproductive performance of infected females are common occurrence in infected dairy herds than in the beef or mixed herds. The fastidious growth pattern of MAP and the absurd response of the host animal immune system to infection have reckoned the diagnosis of JD to be an uphill task. Diagnosis of subclinical infection with MAP in ruminant species remains one of the greatest challenges to JD control, both at the individual-animal (Collins, 1996; Manning & Collins, 2001) and herd-level (Jordan, 1996). High shedders of MAP and animal with clinical signs of JD are responsible for the greater part of the contamination of their environment, the economic damage in infected herds and the presence of bacteria in milk (Grant, 2003). To monitor the progression of a control programme the herds need to be tested. Serology is the most practical method used for this purpose. The enzyme-linked immunosorbent assay (ELISA) is a suitable diagnostic tool to detect serum antibodies against MAP on a large scale, because it is possible to test large numbers of samples with a high reproducibility (Collins, 1996; Van Maanen et al., 2003; Attili et al., 2011). As at now, no effective treatment of this serious infectious bacterial disease is available, and the long-term effect of vaccination, though it reduces the incidence of clinical disease and bacterial shedding, is controversial and fails to eliminate or eradicate the disease in a herd (Rosseels and Huygen, 2008). Thus, the spread of the disease can only be stop through the implementation of rigorous control programmes whereby animals that tested positive for JD are kept separate from other animals or even culled.

Ruminant milk has been described as a potential source through which humans could be infected with MAP (Chiodini & Hermon-Taylor, 1993; Grant, 2003; Ayele et al., 2005; Favila-Humara et al., 2010). The milk could be contaminated with MAP by either direct shedding of the organisms or fecal contamination during or after milking (Grant et al., 2002). Humans are exposed to MAP by direct contact with infected material and in retail milk supplies (Ellingson et al., 2005). Viable MAP has been isolated from milk and colostrum of clinically and subclinically infected cows and ewes (Sweeney et al., 1992) and direct and indirect infection of the mammary gland has been documented in small ruminants (Stehman, 1996; Ngu Ngwa et al., 2010; Attili et al., 2012). MAP has been demonstrated by PCR in goats’ milk from bulk tanks in farms in the UK (Grant et al., 2001) and has a thick, relatively impermeable complex cell wall that is rich in lipids. This confers acid-fast properties and enhances its survival in water and in the environment for long periods thus complicating the eradication of the disease (Whittington et al., 2005). Also, the complex cell wall has been observed to increased MAP resistance to high temperatures when pasteurizing milk (Grant et al., 2005), and this is another virulence factor which may be important in the transmission of MAP to humans. Although, some researchers have demonstrated that effective pasteurization should kill all MAP organisms (Stabel, 2001), viable MAP has been reported in retail
pasteurized milk (Grant et al., 2002; Ayele et al., 2005). The bacterium can survive pasteurisation. Sub pasteurization temperature of milk for cheese production may not be sufficient for complete inactivation of MAP (Pearce et al., 2001; Gao et al., 2002; Stabel & Lambertz, 2004; McDonald et al., 2005; Rademaker et al., 2007) showed that MAP is not completely destroyed in milk artificially contaminated with a high inoculum even after a combination of high pressure (600 MPa) and pasteurization. Cheese production processes have been shown to have little effect on the viability of MAP (Chiodini & Hermon-Taylor, 1993; Sung & Collins, 2000). Furthermore, viable bacteria have been demonstrated in hard and semi-hard cheese 120 days after production (Spahr & Schafroth, 2001). In another dimension, meat products may also serve as a source of MAP transmission to humans (Grant, 2006). The meat products may be contaminated following the dissemination of MAP within the tissues of the infected animals. Of recent, MAP genome was found on beef carcasses after flaying and dressing (Meadus et al., 2008).

2.1 MAP and Crohn’s Disease

MAP has been associated with Crohn's disease (CD) in humans, a chronic inflammatory bowel disease of human gastrointestinal tract, which is characterized by general malaise, chronic weight loss, abdominal pain, and diarrhea (Bull et al., 2003; Abubakar et al., 2007; Feller et al., 2007). Although the distal ileum is most commonly involved, CD may affect any part of the gastrointestinal tract. It is a life-long disease that has no known cure and sufferers may have a poor quality of life because of pain and, in extreme cases, uncontrolled discharge of intestinal content (Hermon-Taylor & Bull 2002). It is believed that CD has a complex and multifactorial etiology.

Crohn's disease could be due to: - a persistent infection, possibly involving mycobacteria (specifically MAP); - a defective mucosal barrier (leaky gut) which allows uptake of bacterial, dietary and other immunogenic macromolecules; - dysregulation of the host immune response with loss of tolerance, aggressive cellular activations and disorders of apoptosis; - genetic susceptibility factors; - a combination of some, or all, of the above (Shanahan et al., 2002). Mutations in a gene on chromosome 16, known as NOD2/CARD15, are associated with Crohn's disease (Hugot et al., 2001). Three major mutations of this gene are most commonly encountered in Crohn's patients – R702W, G908R and L1007 fsinsC (Lesage et al., 2002). The proportion of cases of Crohn's disease that can be attributed to NOD2/CARD15 mutations has been estimated at 15%-30%, which would be consistent with roles for a number of other factors, both genetic and environmental, in the pathogenesis of Crohn's disease. Those data provides compelling evidence to support the hypothesis that Crohn's disease results from bacterial insult in genetically susceptible individuals. NOD2/CARD15 serves a role in bacterial recognition, activating the nuclear factor (NF) κB pathway. Cellular studies have documented that NOD2/CARD15 is a cytosolic protein activated by muramyl dipeptide, a degradation product of bacterial peptidoglycan (Sechi et al., 2005). Since the initial isolations (Chiodini et al., 1986), MAP has been successfully cultured from resected tissue of Crohn's patients in various parts of the world: USA, UK, The Netherlands, Australia, France, Italy and the Czech Republic (Chamberlin et al., 2001; Hermon-Taylor & Bull, 2002; Bull et al., 2003; Naser et al., 2004; Sechi et al. 2005, Feller et al., 2007; Singh et al., 2008); and also from breast milk of Crohn's Disease suffering mothers. This can expose infants to MAP very early in life, a significant concern if they are genetically susceptible to developing Crohn's Disease (Naser et al., 2000). MAP has as well been cultured from the blood of CD patients (Naser et al., 2004). It is also concerning that MAP has been found in milk and meat taken from infected cattle (Mutharia et al., 2010).

However, the zoonotic potential of the organism remains controversial (Chiodini & Rossiter, 1996; Sechi et al., 2005), and the etiology of CD still a mystery despite the significant medical debate that has
taken place over the past years. In a short note, the clinical and histopathological similarities between human chronic granulomatous enteritis, intestinal tuberculosis, and JD obtained from literatures have led to the unsubstantiated belief that *MAP* has an important role in the etiology of CD. Furthermore, there is a broad unanimity that CD results from a sustained immune response that arise from an environmental stimulus in a susceptible host (Shanahan, 2002), and *MAP* has been postulated to be a potential stimulus at least in a subset of patients (Hermon-Taylor & El-Zaatari, 2004).

### 2.2 *MAP* and Diabetes

Evidence supporting a link between *MAP* and T1D includes: higher detection rates of *MAP* by IS 900 specific PCR in samples from T1D patients compared with controls; demonstration of a serological response to *MAP* antigens and whole cell lysates in T1D patients (Rosu et al., 2008). Moreover the founding of a relevant SLC11A1 (ex NRAMP1) gene polymorphisms in T1D patients (Paccagnini et al., 2009) previously reported in Crohn’s patients (Sechi et al., 2006), suggests indeed that there is a strong possibility for *MAP* to be involved with autoimmune responses in T1D just in the same manner as it did in Crohn’s. NRAMP1 (Natural Resistance-Associated Macrophage Protein) is an iron transporter associated with macrophage activation. This gene has multiple pleiotropic effects on macrophage function, including regulation of cytokine production, TNF-α, II-1 b, iNOS and regulation of MHC-II expression and antigen presentation functions (Blackwell et al., 1999; Dai et al., 2009). All of these activities are not only essential for protection against mycobacterial infection (innate defenses), but also critically involved in the induction and progression of autoimmune diseases. Since *MAP* persists within macrophages and it is processed by dendritic cells (DC), mutant forms of SLC11A1 may alter the processing or presentation of *MAP* antigens leading to diabetogenic responses (Dai et al., 2009).

Although evidence for a cause-effect relationship is lacking, *MAP* transmission to humans has long been associated with Crohn’s disease both in Italian people from Sardinia region (Sechi et al., 2005; Di Sabatino et al., 2011) and elsewhere (Naser et al., 2004). The hypothesis stating that *MAP* infection may be a potential candidate environmental trigger also for T1D is based on these key findings: - *MAP* infection is highly prevalent in Sardinian T1D patients; - *MAP* DNA can be evidenced from blood in 63% of Sardinian T1D patients, but only in 16% of healthy controls (Sechi et al., 2008); - *MAP* envelope protein MptD can be detected in the blood of 47.3% Sardinian T1D patients, but in a smaller proportion of T2D patients (7.7%) and healthy controls (12.6%) (Rosu et al., 2009); - *MAP* can be cultured from blood (Rosu et al., 2009); - *MAP* infections triggers a specific humoral response, as Sardinian T1D patients display high frequencies of antibodies (Abs) reacting against mycobacterial proteins (heparin-binding hemagglutinin, glycosyl transferase, whole *MAP* lysates (70% Ab+ T1D patients vs 7.6% Ab+ healthy controls) and the *MAP*-specific proteins MptD like MAP3738c when compared to T2D and healthy controls (Sechi et al., 2008).

The linkage between *MAP* and T1D comes from the concept of molecular mimicry: cross-reactive immune responses because of significant structural homologies shared by molecules encoded by dissimilar genes. Either linear amino acid sequences of the molecules or their conformational epitopes may be shared, even though their origins are separate. The disease pathogenesis may involve multiple factors including genetics of the host, *MAP* strain, activation status of the autoreactive T cells, upregulation of pancreatic MHC class I antigens, molecular mimicry between *MAP* and β cell epitopes and T-cell mediated β-cells destruction by cytotoxic mechanism (Davies, 1997). *MAP*, as one of the environmental factors affecting the induction of T1D, may act as triggering agents of autoimmunity or (less probably) as primary injurious agents, which directly damage pancreatic β cells. Immune responses against a determinant
shared by host cells (β- cells) and MAP could cause a tissue-specific immune response by generation of cytotoxic cross-reactive effector lymphocytes or Abs that recognize self-proteins located on the target cells (van Halteren et al., 2002; Dow, 2006). Notably, it has already been reported as a case of cross-recognition between the mycobacterial heat shock protein 65 (hsp65) and the self auto-antigen, glutamic acid decarboxylase (GAD65), involved in T1D patients (Scheinin et al., 1996). Here it was demonstrated that Abs against MAP 3865c are capable of cross-reacting with host determinants.

It was shown that Sardinian T1D patients specifically mount anti-MAP3865c Abs responses. Two Abs epitopes were identified in the MAP3865c sequence and shown to be homologous to the β-cell antigen zinc transporter 8 (ZnT8) (Chimienti et al., 2004) targeted by auto-Abs in T1D patients (Wenzlau et al., 2007). Anti-MAP Abs recognizing these regions was found to be cross-reactive with the homologous ZnT8 sequences, raising the possibility of a molecular mimicry between mycobacterial and β-cell epitopes. It was successfully demonstrated the presence of MAP’s DNA in T1D Sardinian cases, this goal was achieved through PCR based detection of IS900 insertion element, a specific signature locus of MAP (Sechi et al., 2008). After identifying MAP in the blood of T1D and aiming to understand the host immune responses to MAP, it was designed an immunoassays (indirect ELISA) for the detection of anti-MAP Abs in diabetic patients. ELISA tests, employing sensitive antigenic targets such as HbHa (heparin binding hemagglutinin) and Gsd (glycosyl transferase) proteins, gave encouraging results (Sechi et al., 2008). However, anti-MAP humoral responses corresponding to HbHa and Gsd could not be indicative of an active infection and also since these proteins are encoded by wider range of mycobacteria, this raised an issue of cross-reactivity with tubercle bacilli which could be an issue to deal with the BCG vaccinated individuals. This prompted Sechi et al. (2008) to set up immunoassays including a MAP specific protein, MptD into the battery of antigens. The detection of anti-MAP Abs revealed extremely significant humoral immune responses in T1D patients when compared to T2D and healthy controls (Rosu et al., 2009, Cossu A. et al., 2011).

Final evidence supporting a link between MAP and T1D was presented in terms of culture of MAP bacilli from the blood of two of the T1D patients from Sardinia (Rosu et al., 2009). Summing up, different MAP proteins were shown to be highly recognized in T1D patients and even if some of them were specific of MAP (MAP3738c and MptD) none of them were homologous to human proteins. It was blasted the whole MAP genome against the human genome and it was identified a protein (MAP3865c) not only homologous to human proteins but also specific to β-cells (Znt8). Building up on the above reports documenting a high prevalence of MAP infection and seroreactivity in Sardinian T1D patients (Sechi et al., 2008; Rosu et al., 2008; Paccagnini et al., 2009; Cossu et al., 2011) it was demonstrated that the MAP3865c protein is a target of Ab responses that cross-react with homologous ZnT8 sequences. MAP3865c is a 298 aminoacid 6-membrane-spanning channel which endows MAP with the ability to transport cations through the membrane, an important feature associated with intracellular survival of mycobacteria (Riccardi et al., 2008). ZnT8 is a 369 aminoacid protein which belongs to the cation diffusion facilitator family of highly homologous ZnT (Slc30) proteins. It displays a remarkably similar structure and function, allowing Zn(2+) to accumulate in the insulin granules of pancreatic β-cells. Zn(2+) cations are essential to form hexavalent insulin storage crystals and, eventually, for effective insulin secretion (Wijesekara et al., 2010). The intestinal localization of MAP infection may also favor cross-reactivity with Abs and T cells recognizing ZnT8.

The primary route of MAP infection is fecal-oral and once ingested, the bacterium lodges in to the mucosa associated lymphoid tissue (MALT) of the small intestine. It is then endocytosed by the M cells of Peyer’s patches, which are further phagocytized by intra epithelial macrophages. Indeed, the first en-
counter with beta-cell antigens takes place in pancreatic lymph nodes (Gagneraul et al., 2002), which also drain intestinal tissues (Turley et al., 2005). The intestinal localization of MAP infection may also give reason for the lack of correlation between MAP IS900 DNA and Ab detection. Not all MAP-infected individuals may mount systemic Ab responses detectable in blood, or they may develop Abs against other MAP antigens.

Recently, an important linkage was found between MAP and T1D patients who were free of tuberculosis and CD based on MAP specific DNA and antibody detection analyses (Sechi et al., 2008). On the other hand, T2D has become an epidemic and is extending in the young adult population, adolescents and even, occasionally, in children (Sturnvoll et al., 2005). A number of studies have associated T2D with mycobacteria (Child et al., 1995; Liu et al., 2006). However, the work carried out by Rosu et al (2008) unequivocally refute such speculations through a robust assay that used three different antigen preparations which were shown to have acceptable sensitivity and specificity in case of T1D. They demonstrated that T2D patients do not have significant levels of anti MAP antibodies in contrast to their T1D counterparts and thus put to rest the long driven speculation that MAP is an infectious trigger of T2D. MAP has been isolated from the blood of Sardinian patients affected by T1D but not in those with T2D (Rosu et al., 2009).

2.3 Conclusions

Just because MAP is detected more often in the blood of T1D patients by culture, PCR or ELISA than in control subjects does not necessarily mean that MAP causes T1D. This is certainly evidence of an association (the occurrence at the same time and in the same patient of MAP T1D) but not necessarily of causation (the organism has directly initiated the disease in the patient). There are several possible explanations for the presence of MAP in the blood of T1D patients: it could be an innocent bystander that has merely colonized the host; it could be a secondary infection agent but not causing the disease; or it could be the primary infectious agent and the cause of T1D. Definitive evidence proving a causal relationship between MAP and T1D is not available at present, perhaps it may never be. However, there is evidence suggesting some kind of association between MAP and at least some cases of T1D, so much that the robustness of the link is undeniable.

The most likely candidates as vehicles of transmission of MAP from cattle to humans are milk (and potentially other dairy products), beef and water. It is supposed that the detection of MAP in ruminants’ milk and by-products represents the potential public health risks associated with this pathogen. Thus, the establishment of policies that will aid in curtailing the spread/transmission of MAP and the effects of its persistence in individuals should be encouraged. MAP has been shown to pass into human breast milk (Naser et al., 2000). However, the consumption of infected ruminant’s milk early in life has been an acknowledged risk factor in the occurrence of this disease (Gerstein, 1994; Gimeno & de Souza, 1997). This was based on the observation that children at risk for T1D who were breast fed exclusively for more than six months were less likely to have T1D later in life than similar risk children who were weaned onto cow’s milk-based formula at an earlier age. Behind these studies is the postulate that there is something about ruminant’s milk protein that is an immunologic trigger for T1D and that hydrolysis of the protein will eliminate the trigger phenomenon. Also, the triggering of autoimmune responses in genetically susceptible individuals is believed to be initiated by environmental microorganisms (Knip et al., 2005; Dow, 2006). Furthermore, other potential routes of exposures to this organism such as water, meat, and the environment should be thoroughly investigated and associated risks and preventive measures evaluated and communicated with the public.
To conclude, T1D has only recently been included to the controversy regarding MAP and human disease that has been lingering for close to a century. In regards to the human toll to individuals with T1D, a quick sense of urgency to the MAP/T1D connection should be given an utmost importance.

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