Candidate genes and immune response biology essay

Science, Biology

Type 1 diabetes (T1D), more specifically called " Insulin Dependent Diabetes Mellitus" (IDDM) is an increasingly common autoimmune disorder with particularly high onset in children. T1D is a multifactoral disease, similar to conditions such as asthma and hypertension, with epidemiology of the condition suggesting that both genetic and environmental components can play a role in its development. T1D development involves significant physiological degradation of a vital endogenous process, and the negative effects associated with full disease development are typically widespread. T1D sufferers are tasked with a constant mediation of their intake and glucose levels; and the condition can, in many cases, lead to much more severe complications in the long term, including reduced or severely impaired vision, kidney failure and increased risk of heart attack or stroke. While many of these risks can be mitigated through careful control of blood glucose levels in the body via management of diet and proper execution of insulin replacement therapy (most commonly insulin injections or through use of an insulin pump), T1D is still associated with impaired quality of life, increased risk of more serious physiological complication and a reduced life expectancy. Incidence of T1D is common, and has been getting more prevalent over the last several years – in the U. K. in 2010, known cases of T1D had risen to approximately two hundred and sixty thousand, an increase of one hundred and twenty thousand from just 1996 – of this number, around twenty five thousand are under twenty five years of age. [Diabetes U. K., 2010]Despite long term study, details of the development of T1D are not conclusively known; including, most importantly, the nature of the specific " trigger", whether genetic, viral or chemical in origin, required to initiate true

disease development, and what specifically occurs in this process [Noble & Valdes, 2011; Berhan et al (2011]. However, while certain elements of T1D development have not yet been identified, current methods for disease screening are well defined and widely consistent – usually involving the detection of Islet cell antibodies (ICAs). The development and appearance of ICAs occurs prior to the appearance of phenotypic effects of T1D and can be used as an indicator for an individual's risk of developing the condition. Three autoantibodies commonly used as such indicators are: GAD65 (GADA), a specific structural variant of glutamic acid decarboxylase, encoded by the GAD2 gene; IA-2 (IA-2A), also known as ICA-512, which are autoantibodies of insulinoma antigen 2, and insulin autoantibodies (IAA) [Gardner, 1999]. Recently, an additional ICA has been proposed; autoantibodies of Zinc Transporter 8 (ZnT8A), also known as SlC30A8, present an additional indication of T1D development risk, appearing in serum samples from 63% of all recently diagnosed T1D patients, and in more than a quarter of T1D patients who tested negative for the presence of the three ICA antibodies commonly used in testing, as described in a study carried out by Wenzlau et al (2008). While no strict order of ICAs appears to exist, and no one autoantibody, in any quantity, is a clear indicator of T1D development; the percieved risk of disease development does appear to be more closely associated with quantities of different ICAs detected in samples (most commonly serum samples). Therefore: the biggest disease risk is naturally associated with individuals who actively produce all known ICAs. [Gardner, 1999]Counter to expectation, the majority of cases of T1D are not of familial hereditary origin. Instead, some combination of sporadic genetic changes

and variable environmental contributing effects are thought to be the key to T1D development: for example, according to figures taken by Diabetes U. K. in 2010, around 90% of T1D patients have no close relatives who also suffer from the condition. [Diabetes U. K., 2010]

Genetic Factors

Over time, analysis of T1D has identified the main causative factors of the condition as either genetic or environmental in origin, or some combination of the two; with genetic risk factors being commonly associated with more significant risk of disease development. In terms of genetic risk factors, several genomic loci have been shown to be linked to an increased risk of T1D development. By far the largest contributing factors to risk are two regions of the Human Leuketocyto Antigen (HLA) region, [specifically class II immune response loci found at position p21. 31 on chromosome 6] called HLA-DRB1 and HLA-DQB1; however the specifics of what, within these regions, is the true contributing factor to T1D is unknown [Noble & Valdes, 2011]. Despite this lack of overall specificity, several alleles of the HLA-DRB1 and HLA-DQB1 loci have been defined as risk haplotypes highest, with the highest risk being associated with the haplotypes DR3-DQA1*0501-DQB1*0201 (also known as DR3-DQ2, or just DR3) and DR4- DQA1*0301-DQB1*0302 (known as DR4-DQ8, or just DR4). [Noble et al, 1996; via Noble et al, 2008] The HLA genotype DR3-DR4, subsequently, is present in close to half of children five and younger who develop T1D, as reported by the Diabetes Autoimmunity Study in the Young (DAISY) [Rewers et al, 1996; via Steck & Rewers, 2011]In addition to several identified risk loci within the HLA complex, there are several established candidates genes for which risk

alleles have been identified. These include the gene for insulin (INS – on chromosome 11p5), the CTLA4 gene (found on chromosome 2q33 – codes for Cytotoxic T-Lymphocyte Antigen 4, a down regulator of the immune response, specifically of T cells), the IL2RA gene (found on chromosome 10p15 – codes for the interleukin 2 Receptor Alpha chain subunit for the membrane bound receptor of the immune system cytokine, Interleukin-2) and the PTPN22 gene (found on chromosome 1p13 – codes for Protein tyrosine phosphatase, non-receptor type 22, which has effects on the responsiveness of receptors for both B & T lymphocytes) [Steck & Rewers, 2011]Rates of T1D have been increasing over the last several decades. In Finland, the country with the highest rates of T1D found worldwide, incidence of T1D had more than doubled in the three decades between the late 60s and the late 90s (21. 9 cases per 100, 000 vs. 43 per 100, 000); furthermore, the size of this increase in prevalence being even larger in very young (less than four years old) children (11. 7 per 100, 000 vs 29. 7 per 100, 000) [Karvonen et al, 1999 – obtained via Viskari et al, 2000]. Similarily in Sweden – the country with the second highest reported incidence of T1D cases – rates of T1D in those age fourteen years and younger rose dramatically: from a mean incidence of 21. 6% across these age groups from 1978-1980, to a much larger 43. 9% in the same age groups measured from 2005-2007; showing that T1D incidence in Swedish children has more than doubled in around 25 years. Additionally, it has been shown that T1D is also associated with some increased risk of developing other autoimmune conditions, most notably autoimmune thyroid disease, with a fifteen to thirty percent chance of disease development occurring in T1D individuals [Berhan

et al, 2011]. This higher chance of developing autoimmune thyroid disease in T1D populations is largely due to their shared autoimmunity origins – both are autoimmune conditions and both also show significant development susceptibility with certain HLA alleles, specifically DR4, along with aforementioned candidate genes such as CTLA-4 and PTPN22 [Huber et al, 2008]. In addition: smaller associated risks are observed for conditions such as Addison's disease (0. 5%) or celiac disease (4-9%) [Barker, 2006]The relative incidence risk of T1D associated with HLA risk alleles was examined with reference to age at onset, specifically early onset (less than five years) versus later onset (more than fifteen years) in a study carried out by Valdes et al (2012). This study utilised genotyping data from three different groups of similar individuals from three countries containing populations of European descent (Denmark, USA, Scandanavia), and compared it to data collected from the Type 1 Diabetes Genetics Consortium (T1DGC, as described earlier). This analysis found that the genotypes which seemed to confer highest risk of T1D were variants of the DRB1-DQB1 genotype, a combination which also seemed to increase the chances of earlier onset for the condition in such individuals. However, it should be noted that samples selected for analysis in this report were primarily of European descent, due to higher levels of genetic susceptibility, and therefore are only strictly relevant to other members of this highest risk population. A further study, by Aly et al (2005), presented additional findings on the contributive effects of the high risk genotype DR3/DR4–DQ8, in conjunction with other risk alleles, to the development of T1D. This analysis was done in relatives of T1D sufferers, a group which already feature higher than average probability of

T1D development – with immediate relatives being fifteen times more likely to also develop T1D than if unrelated to a T1D sufferer [Rich et al, 2006]. Relatives who possessed both the high risk genotype DR3/DR4–DQ8, as well as one of two other genotypes: –23 HphI, an SNP of the insulin gene; or PTPN22/LYP, a missense mutation of the PTPN22 gene, were tested for ICAs over either three or five years. Polymorphisms of the insulin or PTPN22 gene, represented in the other two genotypes tested for in this analysis, represent two of the other major genetic risk factors for developing T1D, behind the major risk alleles of the HLA locus. Results found that rates of T1D were increased in relatives with both DR3/DR3-DQ8 and the –23 HphI insulin gene risk genotypes alleles: rates of incidence were 28. 1% for those expressing ICAs and 5. 6% for controls in samples tested over a three year period; incidence rates of 25% for those expressing ICAs and 0% for controls were observed in data sets taken from individuals tested over five years. Risk increase was only shown for samples expressing both DR3/DR3-DQ8 and the –23 HphI insulin gene; no significant increase in risk was associated with samples positive for both DR3/DR4–DQ8 and PTPN22/LYP high risk genotypes; or for any of the PTPN22 genotypes. A large genome wide association study, carried out by Barrett et al (2009), attempted to validate the risk for T1D development associated with several genomic loci, as well as to identify additional loci which can contribute to disease onset. This study involved the meta-analysis of three established, large scale datasets, collectively providing analyses of over sixteen and a half thousand case studies of T1D. These include the T1DGC (the Type 1 Diabetes Genetics Consortium – uses affected sib-pair analysis of around 4000 international

samples {from the UK, Europe, USA and Asia}, testing for elements of T1D [Rich et al, 2006]), the WTCCC (Wellcome Trust Case Control Consortium, for which T1D datasets exist [WTCCC, 2007 & Cooper et al, 2008]) and the GoKinD/NIMH (Genetics of Kidneys in Diabetes, with data from the National Institute of Mental Health being used as reference samples [Cooper et al, 2008]). Inclusive of the established HLA high risk genotypes and the candidate genes described above (INS, CTLA4, PTPN22, IL2RA); the results of the study by Barrett et al (2009) highlight forty one unique disease associated genomic loci. Each loci showed statistical relevance to the development of T1D in the results of the analysis; each presenting different levels of associations with disease risk, and with several being found in established, defined gene regions of the genome. Some of these loci were of gene-coding regions: including the interleukin genes (namely IL2, IL10, IL19, IL20 & IL27), the transcription activator/repressor gene GLIS3, several cluster of differentiation genes, including CD69 & CD226, and IFIH1/MDA5, which encodes a pattern recognition receptor protein (PRR), specifically a RIG-1 like receptor, named after the eponymous RIG-1 PRR protein. PRRs are important components of the adaptive immune system, specifically the part which deals with viral infection; being involved in the recognition of foreign viral agents, such as viruses, via their RNA/RNA based components [Foxman & Iwasaki, 2011]. Analysis such as this helps to further illustrate T1D as a complicated multifactorial genetic disease, for which successful screening and diagnosis will only improve as the knowledge of causative genetic factors increases.

Enteroviral Factors

While genetic risk factors such as those detailed above represent the most significant, and potentially essential, factor in the potential for T1D disease development; compelling evidence has been accrued which supports the link between T1D and infection by viral agents, specifically enteroviruses. Enteroviruses belong to the Picornavirus family, which are small, non enveloped viral agents. There are 60 different subtypes of enterovirus, including the polioviruses, the most well known. Infection with an enterovirus is relatively common with children and young adults, occurring typically in lymphoidal tissues, specifically within the small intestine and pharynx of sufferers. From these sites of origin, the viral agent can spread to other sites, including to the pancreas, and have been observed to cause a variety of symptoms, ranging from relatively mild (rashes, inconsequential fever) to very severe (conditions such as aseptic meningitis and the eponymous Poliomyelitis or polio, the risk of which has now been largely reduced due to vaccination) [Akerblom et al, 2002]A potential link between T1D and enteroviruses was first presented by (Gamble et al, 1969); over a two and a half year period, serum taken from a series of T1D positive patients recently diagnosed with the condition, in comparison with sera of control patients, was found to contain antibodies the enterovirus Coxsackie B (specifically the variant Coxsackie B4) at a significant threshold. This finding was also corroborated, at least in part, by a concurrent study by Gamble & Taylor (1969), which found that seasonal incidence rates of T1D correlated with times of high prevalence of Coxsackie B4 infection, peaking in the late summer to early autumn, with no link detected by the study between onset

of T1D and another infectious agent. Much evidence has been published in support of a proposed connection between enteroviral infection and chance of T1D development: for example, in a meta-analysis by Akerblom et al (2002), results a comparative investigation was carried out of several studies which analysed children who had or would go on to develop T1D for incidence of infection by an enteroviral source. It was found that enteroviral infections in these sample sets correlated with an increased rate of T-Cell proliferation and that an average of 34% (ranges went from 27-64%) of young patients just diagnosed with T1D possessed RNA from an enteroviral source in their blood. By comparison, individuals tested who were not yet T1D: the relative number of patients expressing these same levels of enteroviral RNA in their bloodstream ranged from 0-4%, far lower than what had been recorded for the other sample set [Akerblom et al, 2002]. The DiMe (Childhood diabetes in Finland) research study, designed specifically to test the effect of enteroviral infection on childhood T1D incidence [i. e. before the age of seven] presents findings supporting the connection between T1D development and enteroviral infection: analyses of serum from T1D cases and controls, and from mothers of T1D children during pregnancy and controls, found that levels of antibodies for enteroviral agents were elevated in both pregnant mothers of eventual T1D offspring and in separate studies of children who would go on to develop T1D, with enteroviral infection frequently occurring prior to heightened levels of ICAs in many pre-T1D cases [Hyöty et al, 1995]. Further evidence comes from Yeung et al (2011), who carried out a meta analysis of data from 24 carefully selected separate observational case control studies involving T1D and enteroviral infection, an

analysis which involved data from close to four and a half thousand individuals (4448 in total), collected in a myriad of ways. The results of this systematic overview highlights that these findings, when considered collectively, illustrate the role of enteroviral infection to the onset of T1D as possessing clinical significance – while a link between an infection by an enteroviral agent and onset of T1D cannot be inferred, the likelihood of such an infection having occurred, and the evidence of such an enteroviral infection being present in the pancreatic tissue of a T1D individual, is shown as being more likely by the results of this analysis. Most strikingly, those newly diagnosed with T1D are nine times more likely to have some form of enteroviral infection, while long time T1D sufferers are eleven times more prone to infection by an enteroviral agent that those without the condition. One rather important finding was that of all papers considered in this meta analysis, those with large sample sets (involving over one thousand participants) corroborated completely with the overall findings of the meta analysis, while several studies with much smaller sample sets (some as low as fifty participants) were less supportive – these results could, however, be victim to one of several " small study effects", whereby data selection and publication biases can affect the data published, in such a way that what is presented, while not necessarily incorrect, is not representative of the area it proposes to cover. Certain enterovirus mediated diseases have similarity to T1D, in the behaviour of onset and effects, and may be useful to help understand the potential mechanisms of enteroviral infection in the development of T1D. For example: paralytic polio – the most extensively studied enteroviral disease – is a consequence of poliovirus-induced

destruction of motoneurons of the anterior horn of the spinal cord. Analogous with type 1 diabetes, the cell damage in polio is extremely cell-type specific and accompanied by a local inflammation reaction. Similarities can also be observed in terms of prevalence of the condition in the population vs. perceived viral infection: for example, despite the increase of T1D in the Finnish population, and the perceived connection between enteroviral infection and T1D incidence, as described above; relative rates of infection by enteroviral agents in the general population of Finland were found to be very low in a study by Viskari et al (2000) and following a trend of decreasing prevalence over thirty years between the late 60s and 90s – in sharp contrast to the upward trend of T1D incidence over the same period. The same scenario was also observed for conditions resembling polio over the same period: Viskari et al (2000) describes how rates of poliovirus infection in children have been falling, while at the same time risk of polio-like complications within childhood populations has been increasing, despite this apparent decrease in poliovirus infection rates. In the case of poliovirus, this is likely due to poliovirus infection occurring later in the child’s life, after the period of immune protection granted by maternally derived antibodies has ended. This presents a possible avenue by which enteroviral infectious agents may affect those susceptible to T1D development, after an initial period of resistance has faded. While the specific " trigger" for the initiation of T1D is not conclusively known, several have proposed that enteroviral infectious agents could be responsible for, or at least contribute to, the transition to an active form of the disease. Jun & Yoon (2000), discussed the potential role of viruses in the onset of T1D, presenting findings which were

further refined and expanded in a later collaboration by Jun & Yoon (2002). These reviews present the two most likely routes of transition from T1D risk to full disease state. The first of these is the direct destruction of beta cells either prior to or after and in conjunction with the effect of autoimmunity mediated processes, whereby the viral agents are having direct cytolytic effect on beta cells. The second is that a long lasting or consistent viral infection could contribute to a process of beta cell destruction solely perpetrated by autoimmune components. This could occur in one of two broad ways: firstly, infection could occur to the sites of beta cells, where changes could occur to the beta cells themselves to promote autoimmune destruction, specifically to their antigenic components to make them vulnerable to this action; secondly, the viral agent could cause some alteration to the function of the immune system and its components, including potentially prompting the release of T1D supportive cytokines such as TNF-α, or amplifying the activity of the immune system or through promotion of B-lymphocite production (which in turn can amplify antibody production and/or act to tag beta cells). One of the major stages in the development of T1D is insulitis: the development of a lymphocyte contingent, mainly consisting of CD4+ and CD8+ T lymphocytes along with macrophages, within the islets of Langerhans of the pancreas prior to the full development of T1D. This collection of lymphocytes, or lymphoid infiltrate, causes insulitis, whereby these lymphocytes directly infiltrate the beta cells of the pancreatic Langerhans, causing inflammation. This process has been linked to the eventual destruction of the beta cells, mediating inhibition of insulin production by the pancreas, and subsequently the development of

T1D. What is crucially important about this process of insulitis, however, is that the positioning of these agents within the pancreatic islets is not, in itself, enough to initiate beta cell destruction and subsequently the symptoms of T1D: while all three elements (CD4+ & CD8+ T-cells and macrophages) are considered as being necessary instigators of beta cell autoimmunity, and they can be observed accumulating within islet lesions, no negative effect is initially observed. This means, therefore, that some other agent must activate, or otherwise allow, the destruction of beta cells in the presence of one or more of these elements – this other agent acting as the so called " trigger" of T1D development, as mentioned previously. Once the activation process has occurred, this large contingent of lymphocytes and macrophages which have amassed within the pancreatic islets will activate, commencing the destruction of surrounding beta cells. This process is rapid and widespread, with macrophages taking on the role of Antigen Presenting Cells (APCs), tagging the beta cells for autoimmune destruction by the CD4+ and CD8+ lymphocytes. [Morran, 2008; Estella et al, 2006; Dotta, 2007]The importance of enteroviral agents, specifically Coxsackie B, in the genesis of insulitis is supported by Dotta et al (2007): pancreatic tissue samples, obtained from both T1D positive and control individuals, were tested for infection by Coxsackie B, as well as any dysfunction associated with the beginnings of insulitis in T1D. Initial work found that three of the six samples (numbered 1-3) obtained from T1D patients had already experienced a substantial reduction in numbers of beta cells present. However, through a process of staining for the viral caspid protein Vp-1, it was found that infection by the enteroviral agent Coxsackie B targeted the

beta cells of pancreatic islet tissue, in all three of the remaining samples examined. Furthermore, said viral staining was also seen to correlate in the majority of cells also stained for insulin content – this suggests that coxsackie B infection may localise inside insulin producing cells. Furthermore, experimental evidence highlights a lower level of insulin secretion, if not production, in T1D pancreatic tissue, compared with tissue obtained from control samples. These results were also replicated in vitro: a sample of Coxsackie B isolated from the pancreatic tissue of sample two was used to infect the islets of other samples and the response recorded. Samples infected in this way were found to possess a reduced level of glucose-mediated insulin secretion. In addition, all in vitro samples – infected and control – were tested for levels of certain cytokines, of which only those involved in the process of response to inflammation were detected: Tumour Necrosis Factor Alpha (TNF-α), a product of, among other things, NK cells and macrophages and which is involved in the initiation of inflammation response, and interleukin 10 (IL-10), which is involved in the repression of TNF-alpha. Therefore, in the results of both in vivo and in vitro studies by Dotta et al (2007) enteroviral infection had occurred in tandem with the initiation of a mild form of insulitis – associated mainly with natural killer (NK) immune cells. NK cells are critical members of the innate immune system which target cells with low levels of the cellular surface molecule MHC I, as such cells are deemed to be foreign or " non-self" by the innate immune system, due to such cells being compromised, in this case by a viral agent. Additionally, insulin secreted in response to glucose levels decreased; while actual levels of synthesised insulin, as well as numbers of insulin-producing

beta cells, were preserved, with none of the typical signs of apoptosis of beta cells within analyzed islets being observed. In another study by Tracy et al (2002) and a follow up by Drescher et al (2004) further evidence is given of the potential role of enteroviral infections, specifically coxsackie B, in the onset of insulitis and subsequent development of T1D. Through purposeful inoculation of T1D sensitive NOD [non-obese diabetic] mice with various viral strains of coxsackie B prior to disease development, Tracy et al (2002) attempted to determine if viral infection would spur the development of T1D, instead finding the opposite in their results: mice directly affected by coxsackie B viral infections showed seeming resistance to the development of T1D: this was shown by decreases in incidence of T1D in NOD strain mice compared to control mice (rates were at least halved, being as low as 1/16 the expected rates of incidence in one case). Furthermore, no evidence of residual coxsackie viral infection replicating was observed within analysed islets - an effect that was seen in both younger (less than six weeks) and older (more than six weeks) pre-T1D NOD mice. As NOD mice are a common model system for T1D humans, these results present a possible explanation for the behaviour of similar viral strain infections in T1D susceptible humans. In the follow-up study by Drescher et al (2004) alternative potential causes for the prevention of replication of inoculated coxsackie B viral strains, which would explain their lack of replication in the results shown by Tracy et al (2002), were investigated. Firstly, detection of the CAR (Coxsackie and Adenovirus receptor), required for Coxsackie B infections to have effect and proposed to be absent in the NOD mice used for testing, was positive in the NOD mice strains used prior. Secondly: the role of endogenous interferons,

and their function to disrupt the infectious action of coxsackie B viral agents, had been proposed as an explanation as to why no replication of coxsackie B viral infections was detected in analysis by Tracy et al (2002); to test this, a strain of coxsackie B with the ability to synthesised interleukin-4 was utilised, classed CVB-mIL4 (murine IL-4). Interleukine-4 is an anti-inflammatory cytokine with the ability to modify interferons such as those proposed to be inhibiting the replication of present coxsackie B. NOD mice infected with CVB-mIL4 subsequently displayed both successful infection and replication of this viral strain, showing the distruptous effect of endogenous beta cell interferons on the infectious action of other coxsackie B strains. Upon establishing that coxsackie B, with sufficient modification to the cellular environment, can replicate within islets; further analysis was performed using CVB-mIL4 comparing the inoculation of younger and older NOD mice with the viral strain and measuring the effects. While younger NOD mice infected with replicating CVB-mIL4 reflected the increased resistance to development of T1D as seen in the data published by Tracy et al (2002), older NOD mice showed more rapid development of the condition: in one case, more than double the number of older CVB-mIL4 NOD mice developed full T1D than older control mice, three weeks after infection by the viral strain (7/10 vs. 3/10); in separate experiments, the viral agents themselves were detected and replicating in the islets of NOD mice just prior to their full development of T1D, specifically in regions where insulin was being developed. Given these findings, Dresher et al (2004) propose that coxsackie B infections trigger the development of T1D only after insulitis, which occurs shortly before the full development of T1D. This action is normally prevented

or minimised by the action of interferons and the innate immune system, but likely occurs in a less effective or more delayed fashion during the later stages of insulitis, due to the release of IL-4The role of enteroviral infection in the genesis of T1D is also supported by the findings of Oikarinen et al (2011). The analysis involved a study carried out using data obtained from Finnish children taking part in the national DIPP study (Diabetes Prediction and Prevention), with the enteroviral RNA levels in the blood of children who went on to develop T1D compared against control cases; measurements were made in periods of every three to twelve months and potential progress of T1D development was measured by screening for ICAs, namely GADA, IA-2A & IAA. Sample cases were matched against controls in a nested fashion: from a much larger pool of controls, up to six were chosen for each T1D individual analysed, selected based on a myriad of factors including location, age (within one month), sex and genetic susceptibility, based on HLA-DQ risk loci. The results reveal that presence of enteroviral RNA within the sera samples of children examines confers an increased likelihood of T1D development. Additionally, the peak time for the appearance of enteroviral RNA in analysed serum samples occurred within the six month window prior to that subject’s development of initial ICAs (at this point, more than 15% of T1D cases showed enteroviral RNA, vs. 3. 3% of control cases). Similarily, an investigation by Salminen et al, (2004), attempts to show the effects of enteroviral infection on the development of T1D. Taking serum and stool samples from Finnish children actively taking part in the DIPP study, and analysing these samples for the presence of enteroviral infection. Results from both collective analysis (combined analysis of stool and serum samples)

and analysis of stool results alone were considered, with the prevalence of enteroviral infection shown to be higher in T1D sufferers in both sets of analysis, with rates of enteroviral infection being considerably higher in T1D sufferers in the collective analysis. Analysis utilised the presence of ICAs and enteroviral antibodies to confer time of initial T1D development and time of enteroviral infection, respectively, with new samples being taken every three to six months.

Interaction of Enteroviral and Genetic Factors

Given the potential for enteroviral infectious agents, such as coxsackie B, to be a major contributive factor, even potentially acting as the " trigger" for the onset of T1D, it becomes important to consider the genetic factors which may affect the ability of such enteroviral infections to successfully invade such a host system, and which may affect the voracity of effect of such an infection in relation to T1D development. One example would be a rare polymorphism of the previously described T1D-risk associated candidate gene IFIH1 which is associated with a decreased risk of T1D development. The paper, by Nejentsev et al (2009), discusses the study used: the resequencing of exonic and splice junctions of ten different genes, including IFIH1 and the previously mentioned PTPN22 and IL2RA, from previously analysed DNA of almost one thousand case and control individuals used for T1D analysis, that was then used for association analysis, between T1D and these genes, in more than thirty thousand other test subjects. Most significant among their findings, however, were 4 identified sequence variations for the IFIH1 gene, all of which conferred a resistance against T1D

development. All four allelic variants identified each differ from more common IFIH1 gene alleles by a single, specific SNP, which is responsible for some significant structural variation to the encoded protein product. The positive contribution to T1D disease risk is highly significant – Nejentsev et al (2009) estimate that possession of one of these four allele variants of the IFIH1 gene can cause a twofold reduction in risk of T1D development. The rare gene allele variant of IFIH1 encodes a dysfunctional IFIH1 protein – this, in turn, suggests that the fully functioning IFIH1 gene protein is potentially involved in the development of T1D. A further study, by Liu et al (2009), also supports this: analysis of a sample set of almost 4500 individuals for IFIH1 genotypes found that high levels of the endogenous IFIH1 protein in the bloodstream, meant to recognise " foreign" agents for destruction, were found in individuals with high risk T1D genotypes. Additionally, Liu et al (2009) genotyped SNPs purported to be associated with T1D development by existing GWAS studies; and found several SNPs in linkage disequilibrium with the IFIH1 gene which appear to show significant association with the development of T1D. Further evidence for the potential link between genetic and enteroviral contributive effects comes from Oikarinen et al (2011). Data obtained from the project investigating the prevalence of enteroviral RNA in the bloodstream of children with T1D compared with T1D-negative controls found that risk of showing positive results for enteroviral RNA within analysed sera were significantly higher for individuals with the high risk HLA-DR3-DQ2/DR4-DQ8 genotype. However, this shared prevalence rate does not prove correlation between this risk allele and enteroviral infection: since DR3/DR4 genotypes are the genetic factors most associated with risk; this

comparison may once again be highlighting the link between overall risk of T1D development and enteroviral infection, rather than specifically indicating increased risk of infecting in the presence of this genotype.

Conclusion

T1D is a widespread condition, and one which has displayed increasing incidence in the worldwide population, with rates of incidence raising by close to 50% in the U. K. in a 14 year period concluding in 2010 [Diabetes U. K., 2010]; and raising by more than 50%, specifically in juvenile populations, of both Finland and Sweden as measured over several decades. Given its increasing prevalence and serious effects on health, T1D has long been a target for research, but the precise requirements for disease onset remain unclear. As a multifactoral disease: the causative factors associated with T1D are widespread and interact in a complex and – despite extensive study – still largely undefined manner. At current, the roles of genetic risk loci are seen as critical in the development of T1D, specifically alleles of the HLA locus DR3 & DR4 and, in a lesser sense, those of candidate risk genes: including the gene for insulin, as well as CTLA4, IL2RA & PTPN22; with the number of unique risk loci statistically associated with T1D now numbering higher than 40 [Barrett et al, 2009]. As reported by Aly et al (2005), relatives of T1D sufferers, individuals with an already significant elevated risk of T1D development, had an even greater chance of T1D development if they had multiples of these risk associated loci, specifically related to the HLA loci DR3/DR4 and an INS gene polymorphism. In addition, however, much time has been recently spent on determining the role of environmental agents on

the development of T1D; with a particular focus given to viral infections, most notably enteroviruses, such as Coxsackie B. Enteroviral infection, seen in conditions such as polio, can spread to numerous other tissues from a site of origin, including the pancreas, and cause a variety of complications ranging in severity. While a causative role for enteroviral infection in the onset of T1D has not been confirmed; numerous studies have shown correlation between the presence of enteroviral infection in those who have been recently diagnosed with the disease. A proposed link between enteroviral infection and insulitis, a preliminary stage of T1D development where C and T lymphocytes amass within the pancreatic islets, causing inflammation prior to the initiation of beta cell destruction, is suggested by several sources. Two such studies analysed aspects of T1D development within childhood populations in Finland: correlation between increased likelihood of T1D development and enteroviral infection is supported by Oikarinen et al (2011); with Salminen et al (2004) instead showing the higher prevalence of enteroviral infection within these T1D sufferers compared to controls. Dotta et al, via the examination of samples of pancreas tissue from T1D sufferers and controls, tested for infection by coxsackie B enterovirus – and found that infections both targeted beta cells and occurred in tandem with mild insulitis; and that insulin secretion and numbers of beta cells present were lowered for T1D tissue samples in experiments carried out both in vitro and in vivo. More direct evidence, obtained from the T1D model NOD mice, finds that the infection and replication of coxsackie B is largely inhibited by agents of innate immunity known as interferons, but that their preventative action becomes less efficient closer to full T1D development,

specifically after insulitis; proposed as being a consequence of increased production of IL4, an anti-inflammatory agent which also inhibits the function of interferons. [Tracy et al, 2002; Dresher et al, 2004]. Additionally, the potential interaction between genetic and enteroviral risk factors is suggested by several sources: both Nejentev et al (2011) and Liu et al (2009) found that partial function polymorphisms of the IFIH1 gene, responsible for recognising foreign agents in aide of the immune response, imparted decreased chance of persistent enteroviral infection and halved the risk of T1D development. T1D is a serious condition, and one which is only increasing in prevalence, but little is still known about its developmental origins. Genetic factors, while significant to the development of the disease, are not sufficient to explain the precise nature of the mechanism of T1D onset, as well as the perceived risk of T1D developing. While no conclusive link has yet been observed between them: enteroviral infection and the onset of T1D display an apparent correlation with different studies observing an increased chance of enteroviral infection in T1D and pre-T1D patients; as well as linking the presence of enteriviral infections to outcomes such as earlier onset of the condition and an increased risk of disease development. Many go further, linking infection specifically with the insulitis stage of T1D development, and even hypothesising enteroviral agents as the possible " trigger mechanism" which causes insulitis to transition into the beginnings of beta cell destruction. Despite this, more evidence is needed to confirm the full extent of the role of enteroviruses in T1D development, and whether they can be considered a true contributive risk factor for the condition.