

Chapter 2

Viruses and Autoimmune Diabetes: A History

R. David G. Leslie, Lily Ho-Le, and Huriya Beyan

Certainty: The Ascent of the Gene

The identification of an association between type 1 diabetes and certain genes transformed our understanding of this and other related diseases, including thyroid disease and multiple sclerosis. That type 1 diabetes is genetically determined and was evident from family, twin and genetic studies. The frequency of type 1 diabetes is higher in siblings of diabetic patients (e.g. in UK 6% by age 30) than in the general population (0.4% by age 30) (Field 2002). Of genes implicated in the genetic susceptibility to type 1 diabetes, the most important are in the histocompatibility (HLA) region of chromosome 6 (Kumar et al. 1993); first sought by Singal but then sought successfully by Nerup and Cudworth (Nerup et al. 1974). Such HLA genes predispose to a number of autoimmune diseases including type 1 diabetes (Kumar et al. 1993; Concannon et al. 2005), as demonstrated in both population and family studies (Redondo et al. 2001; Kumar et al. 1993; Meyer and Thomson 2001). Genes encoding HLA molecules and located within the major histocompatibility complex (MHC) on the short arm of chromosome 6 are associated with type 1 diabetes. The MHC complex is a polymorphic gene complex in which multiple alleles exist for each genetic locus. The MHC is divided into class I (HLA-A, -B and -C), class II (HLA-DR, -DQ and -DP) and class III (genes for complement components). The classes I and II proteins coded by the relevant genes are transmembrane cell surface glycoproteins which are critically involved in the presentation of both self- and foreign antigens as short peptides to T-lymphocytes.

R.D.G. Leslie, M.B.B.S., M.R.C.S., M.D. (✉) • L. Ho-Le • H. Beyan
Blizzard Institute, Queen Mary College, University of London, London, UK
e-mail: r.d.g.leslie@qmul.ac.uk

HLA genes are highly polymorphic with a degree of coding region diversity unequalled elsewhere in the genome. Polymorphisms of certain genes probably originated in selection pressures exerted by environmental factors including epidemics, climatic change and availability of food. A non-human somatic study of the HLA DQ beta region suggests that this region has been in balanced polymorphism for ten or more million years (Meyer and Thomson 2001). To maintain the extraordinary diversity of HLA types over this time, selection pressures must have been operating; otherwise most alleles would have been lost through genetic drift. It has been proposed that infectious pathogens are the major cause of HLA diversity. The distribution of sequence variation is clustered in nucleotides which code for amino acids composing the antigen-binding groove. This implies that natural selection must have acted at this binding site to maintain structural diversity for peptide binding. By 1990, it seemed most likely that this peptide-binding site identified an autoantigen, and that the trimolecular complex of HLA, autoantigen and T-cell receptor reflected an autoimmune disease process (Nerup et al. 1974). Such an argument was supported by the impressive technical genetic achievements, using single nucleotide polymorphisms, association studies and genome-wide association studies (GWAS), most notably by Todd and his colleagues (Nejentsev et al. 2009). Allied to which was the comparatively unimpressive ability of epidemiologists to identify any key non-genetic factor.

But the limited degree to which HLA and other non-HLA could account for all the risk of type 1 diabetes, the missing heritability, remained an issue. The term heritability reflects gene expression or penetrance in a given environment. The best estimate of heritability can be obtained by determining concordance rates of twins. Both identical and non-identical twins share the same environment in childhood but only identical twins share the same genes. In the classic twin method the difference between the concordance rates for identical and non-identical twins is doubled to give an index of heritability. Higher concordance rates, for autoimmune diseases in general and type 1 diabetes in particular, in identical compared with non-identical twins are consistent with a genetic influence on these diseases (Salveti et al. 2000). Estimates of heritability can be obtained from studies in Finland and the University of Southern California; in both the estimates are substantially less than 100% which means the disease is unlikely to be autosomal dominant (Hytinen et al. 2003). Age-related genetic factors also influence the risk of type 1 diabetes, as the disease risk is lower in adults than in children, and the range of incidence across European countries is also reduced in older age (Kyvick et al. 2004). Survival analysis estimated that non-diabetic identical twins of probands diagnosed with type 1 diabetes under 25 years of age had, in one study, a 38% probability of developing diabetes compared with only 6% for twins of probands diagnosed later (Salveti et al. 2000). Such a remarkably low twin concordance rate for adult-onset type 1 diabetes implies that the genetic impact in adult-onset type 1 diabetes is limited, and certainly lower than that in childhood-onset disease (Salveti et al. 2000; Hytinen et al. 2003; Kyvick et al. 2004). These effects were widely attributed to a stochastic effect by geneticists, but there remained the possibility that other non-genetically determined effects (such as epigenetic effects or environmental effects) might be important. HLA associations with these diseases could, after all, operate through susceptibility to certain undefined infections.

Uncertainty: Autoimmunity as a Disease Process

Autoimmune diseases are the third leading cause of morbidity and mortality in the developed world, only surpassed by cancer and heart disease. Most autoimmune diseases are thought to be complex disorders involving the interaction of non-genetic, probably environmental, factor(s) with more than one genetic factor. The evidence suggests that for the generality of human autoimmune diseases there is no specific “autoimmune gene” but instead a combination of normal genes and common polymorphisms (e.g. HLA haplotypes) which provide a genetic susceptibility to non-genetic factors with which they interact, resulting in an abnormal autoimmune response (Field 2002). The immune system is designed by nature to protect us from our environment (Janeway 2001). But activation of the immune response not only protects us from disease, as in infectious diseases, but also causes disease, as in autoimmunity. Autoimmunity is important to the fitness of the organism. Most individuals produce autoantibodies and autoreactive T-lymphocytes. However, only about 5% of any population develops an autoimmune disease. Control mechanisms must therefore operate to control the development of autoimmune diseases. These control mechanisms remove cytotoxic immune cells in various ways including: clonal deletion, clonal anergy and limiting antigen accessibility to the immune system. Antigen accessibility is limited by being processed for presentation to the immune system or by autoreactive T-lymphocytes circulating in an inert state. Breakdown in these control mechanisms could lead to disease. Diseases associated with autoimmune phenomena tend to distribute themselves within a spectrum of organ-specific diseases, such as type 1 diabetes and non-organ-specific diseases such as systemic lupus erythematosus. There may be clustering of diseases at either end of this spectrum; thus, T1DM is more common in patients with thyroiditis or adrenalitis. Autoimmunity, in the form of autoantibodies, is common after many infections and may well result from the mimicking of host proteins by antigens of the infectious agent. Autoimmune disease has long been considered as a shadow following infections. Epidemiological evidence shows that rheumatic fever follows streptococcal infection and *Trypanosoma cruzi* infection is the instigator of Chagas’ disease. There is, however, little information regarding the mechanism by which such a train of events is initiated, e.g. there are no certain examples in humans in which molecular mimicry gives rise to autoimmune disease (Table 2.1).

For all that type 1 diabetes is considered an autoimmune disease, we must, therefore, acknowledge that the evidence is incomplete. Rose and Bona defined autoimmune diseases as those that show three features (Rose and Bona 1993): (a) defined

Table 2.1 List of potential environmental agents

General factors	Specific factors
Hygiene	Viruses (e.g. enteroviruses)
Parasites	Bacteria
Co-existent infections (TB or malaria)	Cow’s milk (through early exposure)
	Toxins

Table 2.2 Autoantibodies as predictors of type 1 diabetes

Autoantibodies

-
- Can appear at an early age, even around the time of birth
 - Can precede the clinical onset of diabetes by some years
 - Have variable predictive value depending on the autoantigen recognised
 - Have increasing positive predictive value with increasing numbers
-

autoantigens and autoantibodies must be present; (b) passive transfer of T-lymphocytes (specific or non-specific) must lead to disease development; (c) immunomodulation of subjects with disease must ameliorate symptoms. We know that the first of these is true and that the autoantibodies can predict the disease with a degree of certainty (Table 2.2). Autoantibodies, originally identified in type 1 diabetes by Bottazzo et al. (1974), has been detected to four major autoantigens, glutamic acid decarboxylase (GAD65) by Lernmark and colleagues, tyrosine phosphatase-like molecule (IA-2) by Notkins and colleagues, insulin autoantibodies (IAA) by Palmer and colleagues, and zinc transporter-8 autoantibodies (ZnT8) by Hutton and colleagues in about 90% of newly diagnosed patients with type 1 diabetes (Baekkeskov et al. 1982, 1990; Leslie et al. 2001). However, transfer of disease is ethically unacceptable though a single case has been described of apparent transfer of type 1 diabetes following a bone marrow transplant from a diabetic donor to a non-diabetic recipient (Lampeter et al. 1993). Further, there was rapid destruction of apparently normal islet insulin secretory cells when islets were transplanted from a non-diabetic twin to their diabetic identical co-twin, indicating that the destructive process must be outside the islet, insulin secretory cell specific and retain its cytotoxic memory (Sibley et al. 1985). The immune system is the most likely candidate for such an extra-islet effect. Finally we must remember that we are currently unable to immunomodulate this disease, let alone ameliorate symptoms, though there is some limited evidence that the disease process can be modified, at least from a Phase 2 study, by immunotherapy with alum-formulated GAD65; at the time of going to press, the preliminary results of a Phase 3 study appear disappointing. Further, subjects with newly diagnosed type 1 diabetes given cyclosporine, a modifier of T-cell activation, are more likely to show a transient improvement in metabolic control in the first 2 years post-diagnosis (Feutren et al. 1986). So only the presence of disease predictive autoantibodies and the trials showing the benefit of cyclosporine and alum-GAD65 in T1DM provide strong, but not definitive, support for it being an autoimmune disease.

The Failure of Immunomodulation and Its Implications

The aim of disease prediction is disease prevention. Type 1 diabetes could be prevented by avoiding those environmental factors which cause the disease process (primary prevention); or by modulating the destructive process before the onset of

clinical diabetes (secondary prevention) or by trying to cure the disease process at the time of diagnosis (tertiary prevention). In the last decade, attention has focused on the possibility of immunomodulation as a secondary or tertiary form of prevention of type 1 diabetes.

Secondary Prevention of Type 1 Diabetes

Secondary prevention (that is after disease induction but before clinical diabetes develops) could prevent autoimmune diabetes by (a) protection of insulin secreting cells; (b) rest of insulin secreting cells and (c) immune modulation including antigen-based strategies. This field has been hindered by the extensive use of an animal model, the non-obese diabetic (NOD) mouse, which can be cured of diabetes in many different ways, but which offers little of value to modify human autoimmune diabetes. For example in both BB rats and NOD mice, insulin, a presumed key antigen, given therapeutically, delayed the development of diabetes and insulinitis, but a study of oral insulin in at-risk children, based on such hypothetical immunomodulation, failed (Skyler et al. 2005). Such trial failures are not only disappointing, but they have also highlighted the problem with relying too heavily on an inconsistent animal model. Immunomodulation suddenly looked less promising as a cure option. So, attention switched back to non-genetic effects and the potential of identifying disease-related factors.

Possibility: Non-genetic Events Renascent

The decline in interest in the destructive immune change as a potential target of therapy has switched the focus away from autoimmunity and back to environmental events. Environmental factors have, indeed, been implicated in the aetiology of autoimmune diseases. These factors include, for autoimmune diseases, and possibly atopy, in general: temperate climate, increased hygiene and decreased rates of infection, vaccinations and antibiotics, drugs (methyl donors such as hydralazine), wheat consumption, iodine levels, increasing wealth; and also for type 1 diabetes specifically: gross national product, overcrowding in childhood and virus infections, early exposure to cow's milk, reduced rates or duration of breast feeding and vitamin D and nitrite consumption (Field 2002; Meyer and Thomson 2001). These factors will be discussed in more detail later.

Acknowledgments We wish to thank the funding agencies involved in our studies including the Diabetic Twin Research Trust, Juvenile Diabetes Research Foundation International, The Wellcome Trust, the Joint Research Board of St Bartholomew's and the Royal London Medical College, European Union and Eli Lilly. We also thank Dr S. Paschou for editorial assistance.

References

- Baekkeskov S, Nielsen JH, Marner B, Bilde T, Ludvigsson J, Lernmark A (1982) Autoantibodies in newly diagnosed diabetic children immunoprecipitate human pancreatic islet cell proteins. *Nature* 298(5870):167–169
- Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P (1990) Identification of the 64 K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347:151–156
- Bottazzo GF, Florin-Christensen A, Doniach D (1974) Islet-cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. *Lancet* 2(7892):1279–1283
- Concannon P, Erlich HA, Julier C, Morahan G, Nerup J, Pociot F, Todd JA, Rich SS (2005) Type 1 diabetes genetic consortium. Type 1 diabetes: evidence for susceptibility loci from four genome-wide linkage scans in 1,435 multiplex families. *Diabetes* 54:2992–3001
- Feutren G, Papoz L, Assan R, Viallettes B, Karsenty G, Vexiau P, Du Rostu H, Rodier M, Sirmai J, Lallemand A, et al. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet*. 2:119–124
- Field LL (2002) Genetic linkage and association studies of type 1 diabetes: challenges and rewards. *Diabetologia* 45:21–35
- Hyttinen V, Kaprio J, Kinnunen L, Koskenvuo M, Tuomilehto J (2003) Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs: a nationwide follow-up study. *Diabetes* 52:1052–1055
- Janeway CA (2001) How the immune system works to protect the host from infection: a personal view. *Proc Natl Acad Sci USA* 98:7461–7468
- Kumar D, Gemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, Dwyer JH, Roy-Burman P, Bray GA, Mach TM (1993) North-American twins with IDDM: genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. *Diabetes* 42:1351–1363
- Kyvick KO, Nystrom L, Gorus F, Songini M, Oestman J, Castell C, Green A, Guyrus E, Ionescu-Tirgoviste C, McKinney PA, Michalkova D, Ostrauskas R, Raymond NT (2004) The epidemiology of Type I diabetes mellitus is not the same in young adults as in children. *Diabetologia* 47:377–384
- Lampeter EF, Homberg M, Quabeck K, Schaefer UW, Wernet P, Bertrams J, Grosse-Wilde H, Gries FA, Kolb H (1993) Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. *Lancet* 34:1243–1244
- Leslie D, Lipsky P, Notkins AL (2001) Autoantibodies as predictors of disease. *J Clin Invest* 108(10):1417–1422
- Meyer D, Thomson G (2001) How selection shapes variation of the human major histocompatibility complex: a review. *Ann Hum Genet* 65:1–26
- Nejentsev S, Walker N, Riches D, Egholm M, Todd JA (2009) Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* 324(5925):387–389
- Nerup J, Platz P, Andersen OO, Christy M, Lyngsøe J, Poulsen JE, Ryder LP, Nielsen LS, Thomsen M, Svejgaard A (1974) HLA antigens and diabetes mellitus. *Lancet* 2(7885):864–866
- Redondo MJ, Yu L, Hawa M, Mackenzie T, Pyke DA, Eisenbarth GS, Leslie RDG (2001) Heterogeneity of Type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia* 44:354–362
- Rose NR, Bona C (1993) Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 14:426–430
- Salvetti M, Ristori G, Bompreszi R, Pozzilli P, Leslie RDG (2000) Twins: mirrors of the immune system. *Immunol Today* 21:342–347
- Sibley RK, Sutherland DE, Goetz F, Michael AF (1985) Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. *Lab Invest* 53:132–144
- Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E (2005) Effects of oral insulin in relatives of patients with type 1 diabetes: the diabetes prevention trial—type 1. *Diabetes Care* 28:1068–1076

Diabetes and Viruses

Taylor, K.; Hyöty, H.; Toniolo, A.; Zuckerman, A. (Eds.)

2013, XXIII, 373 p., Hardcover

ISBN: 978-1-4614-4050-5