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Twin Studies in Auto-immune Disease

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Abstract. Immune-mediated diseases affect up to 5% of the population and are a major cause of morbidity and mortality. These diseases can be organ specific, such as insulin-dependent diabetes (IDDM) and non-organ specific, such as Rheumatoid Arthritis (RA). Identical and non-identical twins have been used to establish whether these diseases are determined by genetic or environmental factors. The results of these studies have been collated in a new section of the Mendel Institute in Rome.

Diseases included in these studies included IDDM, RA, Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS) and Myasthenia. Striking differences in concordance rates between identical and non-identical twins in all these studies suggest that genetic factors are important in causing these diseases. All the diseases are known to be associated with HLA genes on chromosome 6 which may account for some or all of the genetic susceptibility. However, in the majority of pairs the affected twin has an unaffected co-twin. These observations suggest that non-genetically determined factors, probably environmental factors and not somatic mutations, are critical. The study of unaffected co-twins, who are at high disease-risk, has allowed the identification of changes which precede and predict the clinical disease. The immune-mediated destruction in many of these diseases is probably caused by T-lymphocytes. Twin studies have shown the importance of genetic factors in determining T-cell responses. Identical twins should, therefore, provide the perfect test bed to assess the role of T-cells in immune-mediated diseases.

Key words: Twins, Auto-immune, T-lymphocytes, Antibodies

This review outlines the potential for twin analysis in the study of immune-mediated diseases. This potential is only now being recognised and should be important in determining the influence of genetic and environmental effects in the cause and complications of these diseases. To encourage this process and collate information obtained from such studies on different diseases around the world we have established a Section for

Immune-Mediated Diseases at the Mendel Institute in Rome with an International Register of such studies

Rationale for studying twins

In the method of analysing twins proposed by Sir Francis Galton, the rates of concordance of a disease for identical and non-identical twins are compared. Both identical and non-identical twins share the same environment in childhood but only identical twins share the same genes. Therefore, identical and non-identical twins will be concordant to the same degree for factors determined by the environment. In contrast, the concordance rates in identical and non-identical twins will differ for genetically determined features. The greater the difference between identical and non-identical twins the more powerful the genetic influence causing a particular feature. In this twin method the difference between the concordance rates for identical and non-identical twins reared together is doubled to give an index of heritability [10]. Heritability reflects gene expression or penetrance in a given environment. Perhaps the best estimate of heritability can be obtained by determining the concordance rate of identical twins reared apart though one cannot, even then, exclude the potentially important influence of a shared environment in utero. Concordance rates are usually expressed as the pairwise concordance ie. $C / C + D$ (where C is the number of pairs concordant and D the number of pairs discordant for the disease). When ascertainment is complete it is possible to calculate the proband concordance ie. $2C / 2C + D$.

There are five reasons for studying twins with a disease:

1) To define heritability: this is defined by the Galton method and requires the study of identical and non-identical twins.

2) As a control group: to define the cause or effect of a disease, other than its genetic background, appropriate control subjects should be matched for factors including genetic susceptibility to that disease. Thus, the ideal control is an unaffected identical twin who has been reared with the index case in childhood.

3) To define non-genetic factors: differences between identical twins must be due to non-genetically determined factors while similarities could be due to shared genetic or non-genetic factors.

4) To define genetic factors: this can be estimated using heritability as outlined above. In addition, altered levels of a factor which is genetically determined should be found in the unaffected identical co-twin of an affected twin when compared with normal control subjects.

5) To define groups at high and low disease-risk: identical co-twins of affected subjects are at high disease-risk whilst for some diseases (eg. IDDM) twins who remain unaffected for several years from the diagnosis of their index twin have a low disease-risk [23].

Nature of autoimmunity

Essential elements of the immune system, such as the T cell receptor and immunoglobulins, are likely to be derived from early recognition molecules and their corresponding

genes. Since the earliest recognition molecules were probably involved in recognition of self, the earliest antibodies were probably autoantibodies. Autoimmunity should not be considered as inevitably deleterious. On the contrary, it is an essential and beneficial mechanism allowing the clearance of self-antigen debris from the circulation and the production of a complex network of immune regulation called the idiotypic network. In short, autoimmunity is important to the fitness of the organism.

Most individuals produce autoantibodies and autoreactive T-lymphocytes. However, only about 5% of any population develop 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 antigen being sequestered in privileged sites such as the brain, by antigen not going 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 insulin-dependent diabetes (IDDM) and non-organ specific diseases such as systemic lupus erythematosus. There may be clustering of diseases at either end of this spectrum; thus, IDDM is more common in patients with thyroiditis or adrenalitis.

Pathogenesis of auto-immune disease

At diagnosis, immune-mediated diseases usually show cellular and humoral immune changes both in the peripheral blood and the target organ. Cellular changes include increased numbers of activated T-lymphocytes, often expressing the HLA-DR antigen on their cell surface [2] and alterations in both the number and function of immunoregulatory T-lymphocytes [29]. Autoantibodies can be detected in up to 100% of patients. The relative contribution of T-lymphocytes and autoantibodies to the disease pathogenesis may vary; in SLE autoantibodies appear to play a major role whilst in IDDM T-lymphocytes are probably critical. In either event it is envisaged that a pivotal role is played by the presentation of antigen in the context of an HLA molecule to a T cell receptor. T-lymphocytes may then invoke an immune response against the target organ. The immune process which damages or destroys the relevant organ can be detected months even years before the inset of any clinical symptoms. The initial clinical sign often suggests compensated organ dysfunction (eg. goitre in Hashimoto's thyroiditis), and then frank organ dysfunction (eg. hypothyroidism). In some conditions the clinical state can vacillate between a compensated and an uncompensated state. Immune changes may also fluctuate according to the stage of the disease process. Changes detected in an acute phase of a disease can disappear in the chronic phase, or during a remission, or following ablation of the antigen source (eg. thyroidectomy for thyrotoxicosis). In some of these diseases immune changes do not presage the onset of clinical symptoms.

Limited role of genetic factors

Genetic factors are important in immune-mediated diseases. Most diseases describe an excess concordance rate in identical as compared with non-identical twins. In population-based studies the concordance rates for identical twins ranged from 12% (RA) to 26% (MS) whilst the concordance rates in non-identical twins ranged from 2% (MS) to 4% (RA) [9,13,14] (Table). It is possible that concordance rates could be higher since these population-based studies have been cross-sectional, and longitudinal studies suggest that concordance rates can increase. For example, in the Finnish study of IDDM the concordance rates were 13% in identical twins while in our British series, which was not population-based but in which follow-up was prolonged, some 36% of non-diabetic cotwins developed IDDM themselves [23]. Nevertheless, concordance rates for identical twins are consistently less than 50%, emphasising the importance of non-genetic factors in the disease pathogenesis. Thus, less than 25% of the variability in the clinical phenotype can be ascribed solely to genetic influences.

Genes in the Class II region of the HLA system on chromosome 6 are associated with many immune-mediated diseases. Current evidence suggests that the HLA DQ region of chromosome 6 is more important than the HLA DR region for some diseases whilst in other diseases such as Ankylosing Spondylitis, the HLA B region is most important [7]. In particular, genes which code for certain amino acids are important; for example, amino acids other than aspartate at position 57 of the DQ beta chains and for arginine in the 52 position of DQ alpha chains are associated with susceptibility to IDDM [15]. Other DQB1 genes, including a gene which codes for aspartate at the 57 position of the DQ beta chain, confer protection to IDDM [26]. The overall structure of the HLA molecule is probably more important than any single aminoacid residue alone. It is possible that genes outside the HLA region play a role in these diseases. There is some evidence that in MS genes in the T-cell receptor (TCR) β region influence disease susceptibility but not genes coding for other TCR regions [4]. The role of these HLA genes as disease susceptibility alleles is limited. The chances of an HLA identical sibling developing IDDM is about 10%, 44 times greater than the normal risk. Such risks are very small when compared with the relative risk of 230 for an identical twin of a diabetic developing IDDM even though only one-third will do so [23]. Since the main candidate for genetic susceptibility to these immune-mediated diseases is in the HLA region which codes for proteins involved in antigen presentation and recognition, the immune response is probably important in the aetiology of immune-mediated diseases.

Importance of non-genetically determined factors

The most powerful evidence that immune-mediated diseases are due to non-genetically determined factors comes from the study of identical twins. As identical twins usually live together in childhood, similarities (concordance) between twins might be due to shared genetic or non-genetic factors. Differences or discordance between identical twins, on the other hand, must be due to non-genetically determined factors. Identical twin studies of immune diseases clearly demonstrate that the majority of cotwins of affected twins do not develop the disease themselves. In population-based studies the

highest concordance rates are of the order of 26%, for MS [9]. In other studies with a longer follow-up but with a more biased ascertainment the concordance rates remain comparatively low — 24% for SLE and about 36% for IDDM (23,8). These studies indicate that the cause of these diseases is likely to be, to a substantial degree, determined by a non-genetically determined effect.

Even identical twins could be genetically different for certain genes. For example, genes could undergo random and ordered rearrangement or mutation and may differ between identical twins [5]. In contrast to antibody genes, genes of the TCR do not appear to undergo somatic hypermutation. If, as is widely believed, T-cells are responsible for the immune destruction of islet β cells then it is unlikely that somatic mutations give rise to IDDM. Several other observations argue against somatic mutations causing the disease. It is unlikely that random somatic mutations could explain the almost constant year-on-year disease incidence within different populations without epidemicity or the lack of evidence of randomness in either the clinical syndrome or the antigens recognised by antibodies in patients with the disease [6]. We therefore believe, though we cannot be certain, that IDDM is not due to a random genetic mutation but is caused by an environmental agent acting in a genetically susceptible individual. Other immune diseases could, however, be due to somatic mutations.

Destructive process is probably immune mediated

It is widely held that the destructive processes involved in causing these diseases are immune mediated. While the destruction of the β cell in IDDM could result from the direct effect of an environmental agent, there is evidence that the destructive process must also involve the immune system. The most striking evidence for this comes from a study of identical twins in Minneapolis [25]. Three insulin-dependent diabetic twins received a segmental pancreas transplantation (without immunosuppression) from their non-diabetic co-twins who were themselves unlikely to develop IDDM because they had been discordant for diabetes for more that 17 years [25]. Diabetes was temporarily cured but then relapsed as the graft stopped functioning. Examination of the pancreatic graft showed that, unlike a graft rejection response, only the β cells were destroyed. The destructive process could not have been inherited since the pancreas came from one of the twins who was not himself diabetic. The rapid destruction of apparently normal β cells when transplanted from one twin to their co-twin suggests that the destructive process must: 1) be outside the islet; 2) be β cell specific and 3) have retained its cytotoxic memory for at least 17 years. The immune system is the most likely candidate for such an extra-islet cell effect. In line with this argument the islets showed an inflammatory infiltrate in which cytotoxic/suppressor T-cells were predominant. It remains possible that the non-diabetic twins' β cells had previously been transformed so that this immune attack need not neccesarily be autoimmune.

Targets of Autoimmunity

In distinguishing between self and non-self the organism's most efficient approach is to identify features (antigens) conserved within a species and not features unique to a sub-

type of that species. Therefore, conserved elements, both structural and functional, are obvious targets for immunity. Organ specific diseases tend to target functional elements such as enzymes, eg. thyroid peroxidase (Hashimoto's thyroiditis) whilst non-organ specific diseases target structural elements eg. histones and DNA (systemic lupus erythematosus).

T-cell response to antigen

The T-cell receptor is composed of two polypeptide chains and linked by disulphide bonds. Each chain is about 40 and 50K in size and contains both constant and variable regions. These α and β chains undergo rearrangement of germline sequences, similar to those of immunoglobulin genes, to generate the diversity of combining sites required to recognise many different antigens. It is proposed that the highly polymorphic variable region contacts antigenic peptide in the HLA antigen binding-groove, an interaction which probably evolved to allow recognition of foreign peptides associated with self HLA so that infected cells could be destroyed. The variable region repertoire of T-cells is likely to be determined to a substantial degree by genetic factors since identical twins show striking similarity for their variable β chain usage [20].

Studies to define the nature of the T-cell repertoire and the T-cell antigen specific response in immune diseases are at a very early stage. TCR usage in immune diseases may not be limited to certain antigens and TCRs. However, in an animal model of MS called experimental allergic encephalomyelitis both a major antigen, myelin basic protein, and dominant TCRs responding to it have been identified [20]. Vaccination with peptides corresponding to a variable $\beta 8$ region of the TCR can prevent disease progression in these animals. It is possible, therefore, that vaccination with modified peptides could block the immune response to critical antigens in other immune diseases and hence prevent progression to disease. In human MS immunodominant epitopes have been identified in myelin basic protein. Differences in the specificity of the immune response between identical twins discordant for MS suggests that the myelin basic protein specific T-cell repertoire can be shaped by non-genetic factors. However, a general lack of differences between identical twins for the frequency of these peptide specific T-cells, their peptide specificity and their HLA restriction cannot account for one twin developing MS and the other not doing so [21,28]. To develop MS other factors may be involved, probably including immunomodulatory mechanisms (eg. cytokines).

Exposure need not lead to disease

The immune changes associated with IDDM do not always lead on to the disease [18]. Increased levels of activated T-lymphocytes have been detected in the majority of non-diabetic identical twins of recently diagnosed diabetic patients who were then followed for up to 10 years without developing diabetes themselves [18]. Autoantibodies to islet cells, insulin, GAD and tryptic fragments of the 64kD islet antigen have also been detected in non-diabetic twins of diabetic patients who, we calculate, are unlikely now to develop diabetes [6]. Islet cell antibodies can remit without progression to diabetes [18,22].

As identical twins can be discordant for these immune changes, namely the production of ICA, GAD antibodies and activation of T-lymphocytes, the changes probably reflect exposure to an environmental event [22]. By implication then, exposure to the critical environmental event which causes these immune changes need not lead on to IDDM.

Evidence now suggests that the islet β cells can be damaged without progression to IDDM. Impaired glucose tolerance was observed in five of 41 identical twins of patients with IDDM, none of whom developed diabetes themselves, and all of whom now have normal glucose tolerance [3]. Some of these non-diabetic twins also had a decreased insulin response to intravenous glucose and an altered insulin to glucose dose-response relationship [19]. Finally, increased peripheral blood levels of proinsulin, the precursor of insulin, have been found in twins many years after the diagnosis of the index case, and when they themselves are unlikely to develop IDDM [11].

Other twin studies have also raised the possibility that immune and clinical changes can occur in the absence of clinical disease. In MS 12 of 24 co-twins of affected twins were found to have changes in their brain on MRI despite the fact that only a fraction of these can be expected to develop MS themselves [16]. Similarly, in SLE 4 of 8 twins of affected patients had antibodies to single-strand DNA without any clinical evidence of disease [13]. We have known for some years that immune changes in organ-orientated diseases, such as thyroiditis, can occur without necessarily leading to destruction of the target cells. It now looks as if the disease process associated with IDDM also encompasses a wide spectrum including immune and metabolic changes which do not lead on to diabetes.

Prolonged pre-clinical prodrome

The evidence suggests that a destructive immune process, once initiated, pursues in some a chronic progressive course. We have detected immune changes up to 14 years before the onset of diabetes [6]. During this prediabetic period we have detected immune, metabolic and clinical changes which herald the onset of clinical diabetes. Twins usually grow at the same rate and to the same final height. However, at diagnosis of diabetes the index twin is often shorter than his unaffected co-twin consistent with growth delay being prevalent in the prediabetic period [12]. Indeed growth delay can occur many months before the diagnosis of diabetes [17].

Twins studied before they developed IDDM already show activation of T-lymphocytes when first ascertained irrespective of the time before they develop diabetes [27]. These changes persist until the diagnosis of diabetes months, even years, later without any consistent alteration in their level [27]. Antigens recognised by serum antibodies in twins tested in the prediabetic period include: islet cell cytoplasmic antigens (ICA), insulin, GAD, a 64kD antigen which incorporates GAD, trypsin derived fragments of this 64kD antigen including 37kD, 40kD and 50kD fragments [6]. Antibodies to all these islet antigens have been found in the non-diabetic twins of patients with IDDM [6]: of 12 twins who developed IDDM subsequently 8 of 9 twins with 37/40kD antibodies and all 6 twins with ICA $>$ 20 JDF units developed diabetes. In summary, the immune response during the prediabetic period involves both cellular and humoral changes probably initiated by an environmental factor and maintained by specific islet

cell antigens. This response persists over a prolonged period up to diagnosis indicating that the process associated with it is not intermittent but continuous.

Metabolic changes can precede the onset of IDDM by many months. Many metabolic changes have been described including changes in glucose metabolism and insulin secretion. The insulin response to intravenous glucose can decline over a number of years even when it is in the normal range [24]. Impairment of glucose tolerance and glucose clearance has been observed many months before the onset of diabetes and before changes in fasting glucose [3]. Impaired glucose tolerance can occur without changes in either other intermediary metabolites or insulin. However, glucose tolerance can improve with time in some twins who do not develop diabetes [3].

Predicting disease

The predictive values of immune changes can be calculated by studying twins who subsequently developed disease as compared with those who are now unlikely to develop the disease as calculated by actuarial analysis. The positive predictive value is determined by calculating the number of twins with an abnormality who develop the disease as a percentage of the overall number of twins with that abnormality. Predictive values for immune or metabolic changes will be different in twins as compared with family or population studies as twins have a greater genetic susceptibility. The risk of developing IDDM if you are the identical cotwin of an IDDM patient is about 36% [23]. The immune changes with the highest predictive value were those induced by environmental events in that identical twins were discordant for them. Antibodies to the 37kD islet protein fragment or ICA with a titre greater than 20 JDF units had the highest positive predictive value (89% and 90% respectively) while GAD antibodies had a positive predictive value of 62% [22]. Antibodies with the highest sensitivity as predictors were to a 50kD fragment and ICA (both 75%). Increased levels of activated T-lymphocytes had a positive predictive value of only 60% but a sensitivity of 100% [27]. The extent and persistence of immune changes is also important in that activation of T-cells with ICA gave a positive predictive value of 90% and their persistence in two consecutive samples a predictive value of 100% [27]. A decrease in the first phase insulin response to intravenous glucose in a group of non-diabetic co-twins of patients with IDDM gave a positive predictive value of 58% [19]. The only other metabolic change of any predictive value is impaired glucose tolerance which gave a value of 33% in a small group of twins [3]. That these predictive values do not reach 100% is testament to the fact that metabolic changes can be detected in twins who are now unlikely to develop IDDM. It remains to be determined whether immune changes in the unaffected twins of patients with other auto-immune diseases can predict the development of the disease.

Differences determining disease

The question arises as to how individuals who develop disease differ from those who do not. A critical role may be played by the molecular complex comprising the HLA molecule, the antigen and the T-cell receptor which is central to the development of an

immune response. The HLA molecules comprise an alpha and a beta chain of amino acids which probably form a "pocket" in which the antigen sits. The nature of the amino acids lining this "pocket", or peptide binding site, could determine the ability of antigen to bind and, by implication, to be presented to the peptide receptor on T-lymphocytes. However, identical twins with the same HLA genes are usally discordant for immune mediated diseases suggesting that factors other than HLA genetic susceptibility must be important. A second essential factor is the nature of the antigen; for example, antibodies to the islet cell are more predictive of IDDM than antibodies to insulin, and high titre antibodies are more predictive than low titre antibodies [27]. Little is known of the third component of the molecular complex, the T-cell receptor. In IDDM, it is the nature, intensity, extent and persistence of changes which determines whether diabetes will develop. The continuing study of identical twins at the molecular level should enable a more sophisticated definition of the differences determining who does and who does not develop a clinical immune-mediated disease.

Table - Concordance rates (%) in population-based studies of immune-mediated diseases

	Identical Twins	Non-Identical Twins
Rheumatoid arthritis	12	4
Multiple sclerosis	26	2
Insulin-dependent diabetes	13	3

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