EDITORIALS

The 64 K question in diabetes

A large body of evidence assembled over the past two decades supports the hypothesis that insulin-dependent diabetes mellitus (IDDM, type I diabetes) is caused by autoimmune destruction of insulin secreting pancreatic β-cells.[1,2] A better understanding of the mechanisms underlying the autoimmune process is important for the prediction and early diagnosis of IDDM, and especially for the long-term goal of a rational immunotherapy to prevent the disease. The well-recognised genetic susceptibility to IDDM appears to be at least partly determined by certain alleles of genes in the major histocompatibility complex which are involved in the presentation of antigens to effector components of the immune system.[1,2] However, it is unclear what might trigger the initial "insult" to the β-cell. A plausible hypothesis is that of molecular mimicry, whereby a foreign antigen (bacterial or viral) provokes an immune response, which cross-reacts with a similar epitope on an endogenous antigen.[1,3] Whatever the mechanism, the β-cell autoantigens that are the targets for autoimmune attack have proved difficult to identify, and have been studied largely by investigating the specificity of antibodies which are present in the circulation of most IDDM patients.[4] There are two potential difficulties with this approach. First, it appears that a cell-mediated mechanism (probably involving T cells and/or macrophages), rather than antibody-mediated immunity, is of greatest importance in the initiation of β-cell destruction.[2,3] Second, at least some of the autoantibodies found in overt diabetics may arise as a response to antigens released from damaged cells—ie, as a consequence rather than a cause of the disease process.

Various components of β-cells have been shown to be autoantigens in IDDM. Insulin itself is the only fully characterised autoantigen,[5] but although insulin autoantibodies may have predictive value they are unlikely to be responsible for β-cell destruction. Antigens exposed on the cell surface, at least transiently, would seem to be the best candidates for involvement in the primary immune attack. A major fraction of human islet cell antibodies appears to be directed against membrane glycolipids.[6] However, specific protein antigens have also been implicated, including a 38 kD protein of the insulin secretory granule membrane which reacts with T-cell clones from an IDDM patient.[7] Another autoantigen that has aroused special interest is a 64 kD protein[8] revealed by immunoprecipitation or immunoblotting of β-cell lysates with diabetic sera. Circulating antibodies to 64 K protein are present not only in human diabetics[9] but also in two animal models of diabetes—the BB rat[10] and the NOD mouse.[11] Although 64 K protein appears to be membrane-associated by some criteria,[12] it may not be exposed at the cell surface.[13,14] Since the identity and function of 64 K protein have remained obscure, its importance in the pathophysiology of IDDM has been difficult to assess.

In this issue (p 583) Jones and Hunter present evidence that 64 K protein is related to the hsp 65 protein family. The term "heat shock protein" (hsp) denotes a growing number of proteins which are synthesised in response to moderately raised temperatures in all organisms studied, from bacteria to man.[15] The increased synthesis of these proteins can be provoked by various stimuli including ethanol, glucose deprivation, steroid hormones, and cytokines,[16,17] so it is more correct to call them stress proteins. That most of these proteins are constitutively expressed and some are among the most abundant cellular proteins suggests they have important functions in normal cells as well as in stress responses. These functions may include assembly and disassembly of protein complexes, and the translocation and degradation of proteins.[15,16] For the present, hsps are classified largely on the basis of size. Proteins of the hsp 65 family have been highly conserved during evolution: bacterial forms have several counterparts in mammals.[18] Bacterial hsp 65 is the major antigen of various infections including tuberculosis, leprosy, syphilis, legionnaire's disease, and also Lyme disease.[17] Moreover, hsp 65 immunoreactivity has been associated with rheumatoid arthritis in man and in rats,[19] which suggests that clinical effects of the disease may be a consequence of an autoimmune reaction to an antigen structurally related to mycobacterial hsp 65. A molecule cross-reactive with hsp 65 of *Mycobacterium tuberculosis* may also be a target antigen in autoimmune NOD mice.[20] It was shown that development of hsp-65-reactive T cells and circulating antibody to hsp 65 correlated with disease progression in diabetic mice. The importance of these responses in pathogenesis was emphasised by the demonstration that injection of bacterial hsp 65 could either induce or prevent development of diabetes depending on the form administered.[20]

Jones and Hunter present two lines of evidence that the endogenous β-cell antigen cross-reactive with

bacterial hsp 65 may be the previously recognised 64 K protein. First, they suggest that 64 K is itself a stress protein, which increases in content in rat insulinoma cells in response to cytokines and heat shock. Second, they report that monoclonal antibodies to *M tuberculosis* hsp 65 react with a 64 K protein in insulinoma cells, and that this reaction is inhibited by diabetic sera containing anti-64-K immunoreactivity. Unfortunately, this latter critical finding is described without experimental details or mention of the controls necessary to establish specificity of the competition between antibodies. Moreover, it was not shown that the reactivity of anti-hsp-65 paralleled that of anti-64-K, in terms of tissue specificity or stress response. The conclusion that there is immunological cross-reaction between bacterial hsp 65 and mammalian 64 K protein, and that 64 K is itself a member of the hsp 65 family, must therefore be regarded as interesting but preliminary. Certainly, the characterisation of autoantigens or epitopes relevant to the pathogenesis of IDDM remains a very worthwhile objective. On such characterisation rest hopes for the treatment or prevention of IDDM by novel therapeutic approaches designed to eliminate specific autoreactive T-cell clones.[3,21]

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PROGRESS IN TUBEROUS SCLEROSIS

Interest in tuberous sclerosis was boosted by the observation in 1987 that a gene locus for the condition might be on chromosome 9 at q34.[1] Although the disease has been recognised since the last century (especially after Bourneville's description of tubers and areas of sclerosis in the brain of some patients with mental retardation and fits[2]) for many years the combination of mental retardation and seizures was a prerequisite for diagnosis. In the 1970s it became more widely recognised that mentally normal parents might have symptomless tuberous sclerosis, and epidemiological studies now indicate that, at worst, 50% of patients will be retarded. Selection bias suggests the true figure is lower than this, and that the birth incidence of the disease may be as high as 1 in 6000. While most cases can be attributed to new mutations, about a third are familial and for such families antenatal diagnosis would be welcome. That it is often difficult to diagnose tuberous sclerosis with certainty in young children and in those with few potential manifestations is a second clinical reason to look forward to isolation of the gene.

Attempts at gene localisation have continued since 1987 and initially the possibility of a gene locus on chromosome 9 was not confirmed.[3,4] The chance discovery[5] of a child with an unbalanced translocation (t11/22q.3q 11.2) resulting in trisomy for the distal part of the long arm of chromosome 11 and tuberous sclerosis suggested a gene locus at 11q23, and studies on a group of American patients with no evidence of linkage to chromosome 9 confirmed this possibility.[6] However, the British families who had initially provided the evidence of a gene locus at 9q34 did not show linkage at 11q23. Could there be two gene sites? A collaborative study combining all available data provided evidence not only for a gene locus at 9q34 but also for genetic heterogeneity.[7] The most likely second location for a gene is at 11q23, but the possibility that there are more than two gene loci must now be entertained. Even the existence of two gene loci means that antenatal diagnosis will not be achieved by use of closely linked markers but must await the discovery of the gene itself, except perhaps in exceedingly large informative families.

So far there has been no suggestion of phenotypic heterogeneity and progress will probably depend on the discovery of more tightly linked markers than those that are currently available. It would also be useful to find other large families for linkage analysis. Clinical studies on such families have highlighted some of the difficulties in diagnosing this disease. Hypomelanic macules (formerly called ash-leaf patches) often cause confusion and can occur in those who do not have the disease.[8] Cardiac rhabdomyomas can be detected with echocardiography but might be confused with papillary muscles; they are at their largest in infancy, when they are most easily seen.[9] Benign rectal polyps have been