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Workshop report

Prevention of IDDM: strategies based on new observations of molecular pathogenesis

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Research into the aetiology and pathogenesis of insulin-dependent diabetes mellitus (IDDM) has progressed rapidly in recent years. Molecular biology, gene technology and immunology have contributed extensively to our knowledge of the interaction between the genetic background, environmental factors and the evolution of the autoimmune mechanisms leading to manifest IDDM. Scientists from 12 countries met in Kirkkonummi, Finland on 6–8 May, 1996 to discuss the prevention of IDDM and to evaluate strategies based on new observations of molecular pathogenesis of the disease, in particular. The following report summarizes the highlights of the presentations.

S. C. Bain (Department of Medicine, Birmingham Heartlands Hospital, Birmingham, UK) presented a review on the genetic models of IDDM. He mentioned that whole genome linkage analysis of IDDM using affected sibling pairs and semi-automated genotyping and data capture is facilitating the genetic dissection of the disease. A major proportion of the familial clustering can be accounted for by sharing of alleles at susceptibility loci in the major histocompatibility complex (termed HLA in humans) on chromosome 6 (IDDM1) and at least 11 other loci. Primary aetiological components of IDDM1 and IDDM2 have been identified; these are the HLA-DQB1 and DRB1 class II immune response genes (IDDM1) and the insulin gene region variable number tandem repeat sequence (IDDM2). Identification of other loci will involve linkage disequilibrium mapping and the sequencing of candidate genes in regions of linkage. – C. Benoist (Illkirch, Université Louis Pasteur, France) stressed that both autoimmune diabetes in mouse and human IDDM are complex diseases in which multiple genetic loci exert an influence on the generation and progression of autoimmunity. He described data obtained with transgenic and mutant lines of mice, which simplify the genetic analysis and allow one to distinguish genetic influences on several distinct stages: generation of the autoimmune repertoire, initial tissue lesions, and end-stage destruction.

Finland is known for its record high incidence of childhood IDDM, and a crucial question in IDDM-associated genetic studies has been to find out whether the genetic background of the Finnish population can explain the high incidence. – J. Ilonen (Turku Immunology Center and Department of Virology, University of Turku, Turku, Finland) presented a summary of the genetic characteristics of the Finnish normal and diabetic populations. The Finns are an "outlier" population in Europe (like the population in Sardinia), characterized by relative isolation and homogeneity caused by recent bottleneck effects which limit the genetic diversity within the population. A low level of "background" polymorphisms helps to single out disease-associated susceptibility genes making the definition of these genes easier compared to mixed populations. The rapid increase of disease incidence since the 1950s must be caused by environmental factors, but the genetic background may still define the extent to which these environmental factors may exert their influence. The frequencies of the known susceptibility genes cannot explain the high disease incidence in Finland, but our understanding is still limited concerning both major susceptibility genes located within the HLA region

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; GAD, glutamic acid decarboxylase; IAA, insulin autoantibodies; ICA, islet cell antibodies; CBV, coxsackie B virus; CM, cow's milk; GADA; FPIR, first phase insulin response.

as well as genes with "minor" effects located on other chromosomes.

The next session focused on autoimmunity and beta-cell destruction. – G. F. Bottazzo (Department of Immunology, The London Hospital Medical College, London, UK) discussed the interaction between genetic and environmental factors in the autoimmune process of the pancreatic beta cells. He stressed the need to get more insight into mechanisms of antigen presentation and co-stimulatory signals, which would enable a more precise understanding of the pathogenesis of the disease. The present concept is that the putative environmental agent induces autoimmunity in the genetically predisposed individual possibly through peptide presentation by HLA molecules. This might occur through a "molecular mimicry" type of mechanism. He proposed that novel epidemiological approaches should be made to identify the real culprit, and described briefly three projects in Sardinia, directed to this aim (the Sardinian Schoolchildren – IDDM, the Sardinian Newborn – IDDM, and the Sardinian Emigrant – IDDM Projects). – L. C. Harrison (and M. C. Honeyman, The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia) described two ways to obtain immunotherapeutic effects with autoantigens. In the first example, i. v. injection of a high dose of autoantigen protein or peptide (proinsulin or glutamic acid decarboxylase (GAD) peptides) into NOD mice resulted in apoptosis of T cells within intra-islet insulitis lesions. It was postulated that the "tolerogenic effect" of high-dose, systemic antigen was due to deletion of antigen-specific T cells mediated by hyperstimulation. The other example of antigen-specific immunotherapy was the weekly administration of insulin by aerosol. The incidence of diabetes in NOD mice was reduced by up to 50%, and was associated with a significant reduction in the severity of diabetes. Insulin aerosol induced regulatory CD8 $\gamma\delta$ T cells, able to block the adoptive transfer of diabetes to young non-diabetic mice. These observations may be relevant to the application of islet autoantigens, in particular (pro)insulin, in the prevention of IDDM in humans at risk.

J. Nerup (Steno Diabetes Center, Gentofte, Denmark) reviewed the present status of the Copenhagen model on the pathogenesis of IDDM, and described his group's work on the molecular characterization and cloning of the genes encoding proteins, in which the cytokine interleukin-1 beta causes consistent and reproducible changes. The Steno group has used two-dimensional high-resolution gels, which allow qualitative and quantitative analysis of protein expression. The perspective of these studies is first to describe at the molecular level the details of the process of beta-cell death, and second to develop rational pharmacological intervention modalities for use in prediabetic subjects for preventing the progression to IDDM. – A. Hänninen (National Public Health Institute, Turku, and MediCity Research Laboratory, University of Turku, Turku, Finland) presented data on adhesion molecules and homing mechanisms in IDDM and in the NOD mouse. Lymphocytes moving into the pancreas during the development of insulitis require adhesion molecules for their emigration from the blood. The group in Turku has characterized the role of vascular addressins, endothelial adhesion molecules which mediate lymphocyte homing to secondary lymphoid tissues, in lymphocytes homing to the pancreas of NOD mice. The findings suggest that diabetogenic lymphocytes are prevalent in gut-associated lymphoid tissue of young NOD mice. – T. Otonkoski (The Children's Hospital and Transplantation Laboratory, University of Helsinki, Helsinki, Finland) described the regulation of beta-cell growth and regeneration. The latter can occur through two distinct processes, proliferation of pre-existing beta cells, and neogenesis of beta cells through differentiation of precursor cells present within the ductal epithelium. The speaker had used human fetal pancreatic cells in in vitro studies on the effect of nicotinamide, the latter being a potent inducer of beta cell differentiation. This effect was specific for the fetal islet stem cells. Another substance, hepatocyte growth factor was identified as a potent beta cell mitogen, and it is proposed to regulate the growth of beta cells during fetal development.

In the discussion it was agreed that more should be learned of the role of adhesion molecules in the infiltration of immunologically active cells into the pancreatic islets, and of the regulation of the replication and differentiation of beta cells.

In the session on putative autoimmune target antigens, E. Bonifacio (Department of Medicine 1, Istituto Scientifico San Raffaele, Milan, Italy) discussed the panorama of autoantigens. The majority of which have been reported as targets of humoral autoimmunity, and a few have also been shown to be recognized by T cells. The large number of putative antigens, including many which are not islet or even neuroendocrine specific, has contributed to speculation that autoimmunity may not be the primary pathogenetic mechanism of disease, but merely a byproduct. Indeed, many of the antibodies reported can only be demonstrated by binding to solid-phase antigen suggesting that they may represent low affinity antibodies with only minor relevance to disease. Only GAD antibodies, antibodies to IA-2 and IA-2 beta, insulin autoantibodies (IAA), and 38k antibodies have been shown to bind antigen in its native state and in liquid phase, and these are now recognized as major islet autoantigens. – S. Kash (and J. Wu, K. Soren, W. Richter, S. Baekkeskov, Departments of Microbiology and Immunology, Medicine and Hormone Research, University of California, San Francisco, Calif, USA,

and I Department of Medicine, University of Ulm, Ulm, Germany) spoke of GAD_{65} autoantibodies in IDDM. They can be found in the sera of up to 80% of prediabetic subjects, and their presence can precede the clinical onset of IDDM by several years. Dr. Kash presented data on ongoing work aiming at identifying disease-relevant epitopes of the GAD molecule. The ultimate goal is to assess possible epitope switching and spreading in the prediabetic period. – A. L. Notkins (and M. S. Lan, Laboratory of Oral Medicine, National Institute of Dental Research, National Institutes of Health, Bethesda, Md., USA) presented his group's studies on the IA-2, IA-2β, and other pancreatic islet cell genes. The first two are members of the transmembrane protein tyrosine phosphatase family, and their intracellular domains are approximately 74% identical. Trypsin treatment of the recombinant molecules revealed that IA-2 is the precursor of the 40 kDa fragment and IA-2β of the 37kDa fragment, respectively. Approximately 70% and 50% of sera from IDDM patients immunoprecipitated IA-2 and IA-2β, respectively. Close to 90% of IDDM sera have autoantibodies to IA-2 and/or GAD_{65}, and it is concluded that IA-2 and IA-2β are major islet cell autoantigens in IDDM, and together with GAD_{65} are responsible for much of the reactivity of classical islet cell antibodies (ICA) detected by immunofluorescence.

H.-M. Dosch (The Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada) presented evidence for p69 being an IDDM-associated autoantigen. p69 is a non-polymorphic cytosolic protein of unknown function with highly conserved primary structure. Its peak expression levels are found in beta cell lines, and considerable levels map to the blood-brain, and testes-blood barriers. p69 was independently cloned through the use of autoantibodies from a diabetic patient and cross-reactive anti-bovine serum albumin (BSA) antibodies from diabetic rats. T cells from newly diabetic children are sensitized to a single epitope ("Tep69") which is fully conserved in human and rodent molecules. Tep69 shows antigenic mimicry with the BSA-derived ABBOS epitope. – M. R. Christie (Department of Medicine, King's College School of Medicine and Dentistry, London, UK) spoke on the characterization of 37 and 40 kDa antigens. These tryptic fragments of islet 64 kDa autoantigens have been identified as major targets of autoantibodies in IDDM. A member of the tyrosine phosphatase family, IA-2 or ICA 512 expressed in islets, was identified as the precursor to the 40 kDa fragments. The 37 kDa fragments are derived from a distinct but related protein, which is another tyrosine phosphatase-like molecule phogrin (also known as IA-2β in some laboratories), sharing a high degree of amino acid sequence similarity with IA-2. Both IA-2 and phogrin are expressed as larger precursor molecules that are processed to 64 kDa glycosylated mature proteins localized in secretory granules of islet cells and some other neuroendocrine tissues. Antibodies to IA-2 and phogrin appear early in prediabetes, in some cases within the first year of life, and are highly predictive of rapid progression to IDDM in a number of populations.

The following session dealt with environmental factors in the aetiology of IDDM. H. Hyöty (and J. Ilonen, M. Knip, H. K. Åkerblom, and P. Leinikki, Turku Immunology Centre and Department of Virology, University of Turku, Turku, Department of Pediatrics, University of Oulu, Oulu, the Children's Hospital, University of Helsinki, and National Public Health Institute, Helsinki, Finland) reviewed the initiation of diabetic autoimmunity by viral triggers. Mumps, coxsackie B viruses (CBV) and congenital rubella are the main suspected viruses. However, eradication of mumps, rubella and measles by MMR mass vaccination in Finland has not led to a decrease in IDDM incidence in children. This indicates that the role of these viruses, if any, is limited to a relatively small proportion of IDDM cases. Recent prospective studies suggest that CBV infections in utero and in childhood can increase the risk of IDDM. Their action may be present in various stages of the process having either an initiating, accelerating or a precipitating effect, and their aetiological fraction may be higher than previously estimated. Repeated hits by various serotypes and interactions with other diabetogenic agents may be needed to produce a cumulative beta cell damage that eventually progresses to clinical IDDM. – G. Dahlquist (Department of Pediatrics, and Epidemiology and Health Research, University of Umeå, Umeå, Sweden) described viral infections and other fetal and neonatal exposures as initiating events for beta cell destruction. She stressed the importance of focusing on the early perinatal period since most cases of IDDM seem to have a slowly progressing beta cell destruction with a long prediabetic period, and tolerance to both exogenous and endogenous antigens is induced in the perinatal period. A linkage of the Swedish Childhood Diabetes Registry and the Swedish Medical Birth Registry showed that perinatal stressful events such as low gestational age, caesarean section, neonatal respiratory disease, and particularly blood group incompatibility were associated with an increase in IDDM risk. Her group also found that children born small for gestational date had a significantly lower risk for IDDM, whereas children born large for date had an increased risk indicating that a high intrauterine growth rate is a risk factor for IDDM. When the mothers carried CBV IgM antibodies at the time of delivery, the odds ratio for the child to develop IDDM was 2.57 (1.02–7.31), the attributable proportion of risk accounting for up to 20% of cases. The speaker stressed the importance of further studying risk factors like

those described above since they may provide a basis for primary prevention of childhood IDDM.

O. Vaarala (The Children's Hospital, University of Helsinki, and the Department of Biochemistry, National Public Health Institute, Helsinki, Finland) reviewed the development of immune response to oral antigens. Oral administration of an antigen may induce systemic immune response or it may tolerize to the antigen. The speaker's group has shown that feeding of infants with cow's milk (CM) formula induces systemic humoral and cellular immune response to CM proteins. The responses later decline suggesting that continuous feeding leads to the development of oral tolerance. In children with newly diagnosed IDDM, the cellular reactivity to CM β-lactoglobulin occurs more often than in healthy children. This suggests that the development of oral tolerance is disturbed in IDDM. To study the possible role of the gut immune system in IDDM, the group tested whether lymphocytes reacting with islet cell antigen GAD_{65} express the $\alpha_4\beta_7$-integrin, which is a homing receptor for the gut-associated endothelial adhesion molecule, MAdCAM-1. Depletion of lymphocytes with high expression of $\alpha_4\beta_7$-integrin resulted in a marked decrease (> 30 %) in the cellular response against GAD_{65} in four of seven patients with IDDM. These findings demonstrate that islet cell reactive lymphocytes express the gut-specific homing receptor $\alpha_4\beta_7$-integrin, which emphasizes the role of gut immunity in IDDM. – R. B. Elliott (and J. Hill, Department of Pediatrics, University of Auckland, and New Zealand Dairy Research Institute, Auckland, New Zealand) discussed the role of CM proteins as diabetogenic agents in experimental animals and in man. Antibodies to various protein components of CM (BSA, β-lactoglobulin, caseins) have been found to be present at higher levels in children with diabetes at diagnosis than in appropriate control subjects. In the NOD mouse only the curd protein, casein appears to be diabetogenic when fed from weaning. The whey proteins, including β-lactoglobulin and BSA, are inactive. Of the two main beta casein variants A1 and A2, only A1 appears to be diabetogenic. A1 beta casein is a relatively modern mutant in dairy herds, and has become the predominant casein in the last three decades in dairy countries. A1 beta casein yields β-casomorphin-7 after intestinal digestion, whereas the A2 variant does so poorly. This peptide binds to macrophages at a different site to two closely related beta casein immunostimulatory hexapeptides, PGPIHN (A1) and PGPIPN (A2), and inhibits the macrophage oxidative burst stimulated by these peptides. It is thus likely that β-casomorphin-7 yielded by intestinal digestion of A1 beta casein influences the activity of gut-associated macropahges. It is possible that the modern change of cow type to A1 beta casein producers from the archetypical A2 producers may be associated with a different pattern of gut-associated peripheral tolerance, which increases the risk of IDDM in genetically prone individuals when this milk is introduced in early life.

In the session on the current status of the diagnosis of prediabetes, M. Knip (Department of Pediatrics, University of Oulu, Oulu, Finland) described the natural course of pre-IDDM. The beta cell destructive process probably progresses step-by-step rather than linearly. It may be clinically meaningful to stage the prediabetic state into early, advanced and late prediabetes, since the risk of progression to clinical disease is directly related to the actual stage. There was a strong correlation between the incidence of IDDM and the frequency of ICA among unaffected children in an international comparison including Finland and Estonia in addition to four other countries indicating that the same proportion of ICA-positive children progress to clinical disease in various countries. There is no predetermined order of emergence of various disease-associated autoantibodies in the prediabetic period implying that none of the presently known autoantigens is the primary autoantigen in human IDDM, if such an autoantigen exists at all. – A.-G. Ziegler (Diabetes Research Institute, Academic Hospital Schwabing, Munich, Germany) described her study on islet cell specific autoantibodies in a follow-up series of a high-risk cohort. In this "BABY-DIAB" project, children of mothers with IDDM or gestational diabetes and of fathers with IDDM, the temporal sequence of the appearance of ICA, IAA, GADA and IA-2 is observed from birth. Of 1019 children included at birth, 513 have been currently followed up to the age of 9 months, 214 to the age of 2 years and 37 to the age of 5 years. At birth, all antibody specificities were frequent in newborns of diabetic mothers, but not fathers, and are suggested to be transplacentally acquired as they are strongly correlated with antibody levels in their diabetic mothers. In early childhood, antibody levels were below the 99th percentile of control subjects in the majority of children. However, 37 children exhibited elevated antibody levels and these were most frequently detected at the age of 2 years. Children of diabetic fathers were positive for at least one antibody more frequently than children of diabetic mothers. There was no specific sequence in the appearance of positive autoantibodies. The presence of multiple antibodies confers high risk for the development of future diabetes, and two of six children who exhibited positive antibody responses to all four antibodies tested, developed clinical diabetes at the age of 13 and 21 months.

G. J. Bruining (and M. R. Batstra, A. van Driel, and H.-J. Aanstoot, Sophia Children's Hospital, Erasmus University Medical Center, Rotterdam, the Netherlands) discussed the question of antibody positivity or negativity. The relation between titre and risk for

diabetes has not been established for GADA. Non-bimodal distributions of GADA were found, in 1403 schoolchildren (age 10–12 years) and 1288 (age 6–89 years) random individuals who were tested for GADA. Cut-off levels based on different criteria, resulting in inclusion or exclusion of individuals, are a potential problem in intervention trials. The assay system proved to be highly stable, but technical aberrations of the 96-well plates sometimes required retesting (2/50 plates). It was concluded that inclusion of controls in every plate is necessary. It is known that about 50% of patients remain GADA positive for many years after the clinical onset of IDDM, while ICAs are usually not detected beyond 3 years after clinical onset. The group in Rotterdam detected GADAs in 20 out of 40 individuals with a mean diabetes duration of 21 years (range 2–45 years). Significant variability in titres within individuals and fluctuations between positive and negative titres were observed over time. The speaker concluded that assay conditions need further standardization. Not only are standard sera required, but a quality assurance programme, a basic tool in clinical chemistry, needs to be developed for GADA assays. More studies are needed on the definition of GADA positivity and negativity. – C. Wasserfall (College of Medicine, Department of Pathology and Laboratory Medicine, University of Florida, Gainesville, Fla., USA) discussed the Gainesville experience of predicting IDDM. He emphasized that ICA are still the most sensitive single predictive marker in first degree relatives, but that combinations of two or more autoantibodies provide increased predictive value. He also pointed out that the sensitivity of various autoantibodies is age-related with a higher sensitivity for IAA and IA-2A in younger individuals, while GADA are most sensitive in adults. – D. J. Becker (Children's Hospital of Pittsburgh, Division of Endocrinology, Pittsburgh, Penn., USA) described the Pittsburgh study on HLA-DQ genotypes and metabolic markers in the prediction of IDDM. The majority of ICA positive first degree relatives who converted to clinical IDDM had high-risk HLA-DQ markers (approximately 50% developed IDDM over a 10-year period by life-table analysis). HLA-DQ markers were better predictors than the presence of decreased first-phase insulin response (FPIR) (30% conversion to IDDM). In subjects with low FPIRs, the addition of high-risk DQ markers improved the positive prediction to 50% and the addition of GAD and/or IA2 antibodies improved this further to 80%. Those subjects with low FPIR and no IDDM frequently had very high insulin sensitivity. She suggested that insulin secretion should be assessed in relation to insulin sensitivity in order to make this metabolic marker a better risk predictor.

The final session was devoted to the prevention of IDDM. As an introduction, H. Kolb (Diabetes Research Institute at the Heinrich-Heine-University of Düsseldorf, Düsseldorf, Germany) reviewed the means to arrest beta cell damage and initiate reparative mechanisms in the beta cell. For intervention in (pre) IDDM basically three targets exist. The first is the genetic predisposition, to be altered by gene therapy. The second target is the beta cell and the third approach targets the immune cell. With beta cells as target one method is to improve the resistance towards inflammatory mediators. This can be brought about by inducing stress proteins in islet cells or by preventing the loss of intracellular NAD via down regulation of poly (ADP) ribose polymerase activity. Also exogenous insulin reduces the metabolic stress of beta cells and thereby causes less antigen expression. Indeed, the speaker's group found insulin treatment to down-regulate inflammatory cytokines in the BB rat pancreas. With the immune system as target, the approach of modulating the Th1/Th2 ratio carries much hope. Candidate treatment protocols such as oral administration of insulin or vaccinations with bacterial compounds appear to work via this mechanism as judged from analysis of cytokine expression in pancreatic lesions.

Thereafter the progress and stage of three prevention studies were briefly presented. H. K. Åkerblom (the Children's Hospital, University of Helsinki, Helsinki, Finland) reported on the nutritional prevention study, comprising the elimination of dietary CM proteins in high-risk infants for the first 6–8 months of life, the study being thus a primary prevention approach. The second pilot study is now progressing, aimed at elucidating the appearance of immunological markers of IDDM over the first 2 years of life. Two secondary prevention studies were described, E. A. M. Gale (Department of Diabetes and Immunogenetics, St. Bartholomew's Hospital, London, UK) reported first on the European Nicotinamide Diabetes Prevention Trial (ENDIT), the target population being high-risk (ICA-positive) first degree relatives of IDDM subjects. Another approach, targeting individuals at genetic risk for IDDM in an unselected population was described by O. Simell (and the Diabetes Prediction and Prevention project, DIPP, Department of Pediatrics, University of Turku, Turku, Finland). Cord blood samples are used for genetic screening, and children at genetic risk are followed at 3 to 6 month intervals for development of islet autoimmunity. Children who in two consecutive samples are ICA positive and over 1 year of age are randomized to receive roughly 1 IU insulin or placebo/kg intranasally once daily as preventive therapy. – In the discussion it was agreed that the first indications whether IDDM is a preventable disease with the tools in testing will be available at the beginning of the next decade.

The last presentation was on an important issue, the psychological aspects in prevention trials, given

by B. Weber (Virchow-Klinikum der Humboldt-Universität zu Berlin, Berlin, Germany). The speaker stressed that the manifestation of a chronic disorder, which potentially endangers the physical integrity of the individuals and limits their life expectancy and quality of life, but also impairs their spontaneity by demanding treatment regimens, may greatly influence emotional stability. Similarly, the detection of susceptibility to such a disease and a high likelihood for its manifestation, possibly also the nature and prospective duration of measures aimed at preventing the disease, may greatly influence an individual's emotional equilibrium. These conditions now exist in first-degree relatives of patients with IDDM, themselves facing the potential of future disease and more or less promising prevention strategies. Apart from the above-mentioned disease-related anxieties and individual, familial, and social influences on both the perception of the threat and strategies to cope with it, scientific conditions such as a placebo-control of intervention measures, providing uncertainty instead of hope about one's own benefit may considerably modify the individual's reactions. Therefore, prevention trials should be accompanied from the beginning by a conscientious psychological supervision of probands for the scientific reason of better understanding the impact of this new field of medical intervention on the individual, but also for providing a network of support for those unable to cope with this challenge.

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