culture modulation of EGFR expression by hormones such as parathyroid-related peptide and retinoic acid or toxic substances such as smoke derived products interferes with placental endocrine functions. Interestingly, in microvilli purified from placenta of intrauterine growth retardation (IUGR) a decrease or absence of the EGFR tyrosine kinase activity is observed. This can be related to a decrease in EGFR expression in placenta with IUGR related to maternal toxemia. In some placentas with idiopathic IUGR a truncated form of EGFR lacking in tyrosine kinase activity, is observed. This suggests that an alteration of EGFR biological activity might interfere with the fetoplacental unit development.

IDDM: Etiology, Genetics and Environmental Factors

31

IMMUNOGENETICS OF TYPE I DIABETES MELLITUS. **M. Trucco**, Department of Pediatrics, University of Pittsburgh, Pittsburgh, PA 15213, USA

Type I (insulin-dependent) diabetes mellitus (IDDM), is generally considered to be an autoimmune disease based on: 1) the finding of numerous inflammatory cells in the islets of Langerhans at the onset of the disease; 2) the presence of circulating autoantibodies directed against the islet cells; and 3) the fact that immunosuppressive agents, such as cyclosporin A, are able to transiently block the course of the disease. Moreover, the observation that certain HLA class II alleles are associated with an increased risk for the disease, supports an autoimmune etiology for IDDM. In particular, the absence of aspartic acid in position 57 of the HLA-DQ β and the presence of arginine in position 52 of the DQ α chain together, have been found to be strongly associated with IDDM in population studies. These heterodimers have been thought to play an important role in the pathogenesis of IDDM. In fact, the HLA class II molecule participates in a complex interaction between the processed foreign antigen and the T cell receptor (TCR) molecule, which starts the immune response. As the result of a stochastic rearrangement among variable (V), diversity (D), joining (J) and constant (C) gene segments encoding both α and β chains, the TCR may assume 10^{10} different configurations. If IDDM is provoked by a single antigen, a restricted set of T cell clones should emerge during the destruction of the insulin producing β cells. However, this phenomenon is difficult to study because T cells from the pancreas of patients with IDDM, at the onset of the disease, are required to characterize this process. We had the opportunity to study the pancreas of a newly diagnosed child with IDDM who unfortunately died of brain swelling during treatment of diabetic ketoacidosis. T cells from isolated islets were characterized based on their expression of different TCR V β regions. The presence of a remarkably restricted TCR V β repertoire of the T cells infiltrating the pancreatic islets of this patient strongly suggests the involvement of superantigens in the etiology of IDDM.

32

POPULATION SCREENING FOR RISK OF IDDM. **E.A.M. Gale**, Department of Diabetes & Metabolism, St. Bartholomew's Hospital, London EC1, UK.

Risk of progression to IDDM has been assessed extensively in first degree relatives of patients with IDDM and highly specific prediction is possible within a small subset of this population. Since around 90% of future cases will come from those who have no close relative with IDDM, prediction and intervention within the general population will become the main priority for the future. Risk of progression to IDDM can be assessed by means of a decision tree analysis. This highlights the different prognosis of markers when applied to those with and without a family history of the disease, and provides a logical approach to disease prediction. Large numbers of first degree relatives must be screened in order to recruit sufficient numbers of high risk individuals for prospective study or controlled trials of intervention. This implies the need for careful standardization and multicentre collaborative studies. This approach should allow new predictive markers and models to be evaluated, and strategies of intervention to be tested, with maximum efficiency and minimal delay.

33

INCIDENCE VARIATION AND ETIOLOGY OF TYPE I DIABETES

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Variations in incidence rates of IDDM have been documented for the last 20 years in terms of geographical heterogeneity, seasonality, age and secular trend. None of these variations can fit an explanatory model for the etiology of the disease but speculations involving "environmental factors" -in contrast with the genetic determinants- originate from these observations.

The geographical variations in IDDM incidence in children across Europe are known since the '70s. They have been claimed to figure a north-south gradient of incidence along the continent, where Finland represented the top and France the bottom. But not

in all European countries was IDDM incidence evaluated and the methodology was so different that it did not allow for a strict comparison. This was the aim of the Eurodiab ACE program, a concerted action by the European Community, to measure IDDM incidence rates in children (0-14yr) with a common, prospective and exhaustive protocol over 2 years (1989-90) to allow for comparisons between geographical area. More than 15 countries are involved in the project and the results demonstrate large (tenfold) variations in incidence rates across Europe: Finland maintains the highest rate worldwide ($\approx 40/10^5$ /yr), followed by the other Nordic countries. There is a trend for the rates to decrease from north to south, but also from west to east: Greece and Romania exhibit the lowest rates in Europe ($< 6/10^5$ /yr). There is the puzzling cluster of Sardinia in the middle of the mediterranean basin with a value as high as in Finland.

The large number of newly diagnosed cases (> 3000) included in the first phase of the Eurodiab ACE program allows for a detailed specific analysis of age and seasonality. Altogether there is an over-representation of boys vs girls (B/G = 1.1). The age distribution at diagnosis was 18%, 34% and 48% in the 0-4yr, 5-9yr and 10-14 yr old groups respectively. There is no significant effect of sex, age group and country by themselves on specific incidence rates, but there is a significant interaction between age group and geographical area indicating that incidence rates for the 0-4 and 5-9 yr groups seem to vary from country to country in a similar fashion but not in the pubertal age group (10-14yr). The observed data for seasonal variation fit to a sinusoidal model with a peak at wintertime, amplitude of which can afford up to 40% of variation and tends to increase with age and with the incidence level of the country.

In the countries with long-term incidence registries it is clear that a secular increase (20-25%) in incidence rates has occurred between the '70s and the late '80s. This is the strongest evidence for the so-called environmental factors to play an important role in the etiology of IDDM. For instance, cow milk consumption has been involved in both geographical and secular variations in incidence of the disease. Large case-control surveys in Sweden have also suggested the role of nitrosamine consumption and of peri-natal events.

Although not largely documented, the geographical distribution of IDDM incidence does not overlap the distribution of the known genetic susceptibility markers linked to HLA DR or DQ in the background populations. Islet cells antibodies prevalence rates in schoolchildren have been measured in several European countries and seem to parallel the incidence of the disease. This type of results needs to be confirmed by very large scaled studies of uniform methodology. This is now the scientific goal of Eurodiab ACE to set up large epidemiological programs across Europe to determine the respective roles of immunogenetic determinants and environmental factors in the etiology of IDDM in children.

Growth Hormone

34

MOLECULAR BASIS OF GROWTH HORMONE ACTION. **C. Carter-Su**, X. Wang, G. S. Campbell, L. S. Argetsinger, N. Billestrup, G. Norstedt, D. Meyer and J. Schwartz, Department of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA; Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark; Center for Biotechnology, Karolinska Institute, Novum, 141 57, Huddinge, Sweden.

The intracellular pathways by which binding of GH to its receptor elicits its diverse effects on growth, differentiation and metabolism have eluded investigators for many years. We have hypothesized that activation by GH of a GH receptor (GHR)-associated tyrosine kinase is an important early, and perhaps, initiating step in signal transduction by GH. This was suggested by initial studies showing that GH stimulates the tyrosyl phosphorylation of the GHR and that highly purified GH-GHR complexes have tyrosine kinase activity. These findings were consistent with GHR itself being a ligand-activated tyrosine kinase like the receptors for many growth factors. However, more recent studies using cells transfected with the cloned liver GHR cDNA lead to the hypothesis that the GHR forms a complex with a non-receptor tyrosine kinase, the amount of which may vary with cell type. Experiments using truncated GHRs expressed in Chinese hamster ovary (CHO) and rat insulinoma (RIN) cells indicate that the kinase is likely to be a ~ 120 -kDa protein. The physiological importance of the GHR-associated kinase is attested to by experiments using anti-phosphotyrosine antibodies, tyrosine kinase inhibitors, and/or truncated and mutated GHRs. The results of these experiments indicate that the GHR-associated tyrosine kinase: 1) is stimulated very rapidly following binding of GH to its receptor (< 30 sec); 2) is stimulated by very low GH concentrations (0.5 ng/ml); and 3) is likely to play a role in stimulation by GH of a variety of cellular responses, including MAP (mitogen activated protein) kinase activity and *c-fos* gene expression. Furthermore, phosphorylation of specific tyrosyl residues in GHR appears to be necessary for some responses to GH, including stimulation of MAP kinase activity. These results suggest that the GHR-associated tyrosine kinase has at least two roles. The first is to phosphorylate and thereby activate other proteins. The second is to phosphorylate tyrosyl residues in itself and the GHR. These phosphorylated tyrosines may serve as docking sites for proteins in other signalling pathways. This new vision of how GH functions should lead to the identification of new cellular actions for GH and thereby increase our understanding of how GH regulates growth, differentiation and metabolism.