PLENARY SESSIONS

Lawson Wilkins Lecture: Diabetes Mellitus, Type I—Recent Advances in Pathogenesis

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ISLET CELL DESTRUCTION AND GROWTH STUDIED IN TRANSGENIC ANIMAL MODELS .
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We have produced and studied several transgenic models of insulin dependent diabetes mellitus (IDDM). For understanding the etiology of IDDM, we have investigated the consequences of expression of host defense molecules in the pancreatic islets. IFN-γ is produced in response to infection and has immune-stimulatory and proliferative activities. To investigate the potential role of IFN-γ in inflammatory autoimmune diseases, transgenic mice expressing IFN-γ in pancreatic beta cells were created. These mice suffer from islet cell loss following the appearance of increasing numbers of lymphocytes within and surrounding the islets. We have studied the islet destruction in these mice and have demonstrated that it is mediated by inflammatory cells. Interestingly a proliferative/regenerative response opposes the lymphocyte destruction. We have recently been characterizing this proliferative axis in the transgenic pancreas. We have acquired evidence that it is initiated by duct cell proliferation and with the appearance of more primitive neuroendocrine progenitor cells along the apical regions of the ducts. The regenerative process in the ins-IFN-γ transgenic mice appears similar to the events that occur during embryonic islet cell development. These studies underscore the lymphokine's ability to initiate a complex "transdifferentiation" pathway within a terminally differentiated structure. Another fascinating molecule with potential relevance for treatment of IDDM is the cytokine IL-10. This molecule appears to have immuno-inhibitory effects that include the suppression of antigen presentation by macrophages. We have recently investigated the effects of the cytokine IL-10 on the pancreatic islets. These efforts were designed to test whether the expression of IL-10 could allow the creation of neutral islet tissue for allografting. Our work demonstrates that this molecule has both the ability to attract lymphocytes in vivo as well as to suppress the anti-islet immune response. We have utilized several experimental protocols to study the in vivo effects of this molecule. The results of these studies argue that this cytokine could be relevant for therapeutic intervention in IDDM. Our methodologies should clarify the potentially pathogenic capabilities of host defense molecules when expressed in vivo. Additional IDDM models, produced by ourselves as well as our colleagues will be discussed.

Transcription Factors, Gene Regulation, and Pituitary Hormone Deficiencies

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PIT-1 AND ITS NATURALLY OCCURRING GENETIC MUTATIONS; ANSWERS AND QUESTIONS ABOUT THE MOLECULAR BIOLOGY OF PITUITARY DEVELOPMENT AND DISEASE. Holly A. Ingraham, Reproductive Endocrinology Center, University of California, San Francisco, California, USA.

We have identified a transcription factor called Pit-1 that is required for establishment of specific pituitary cell types in normal development [1,2]. Pit-1 expression is restricted to the subset of cells producing growth hormone (GH), prolactin (PRL) and thyrotropin (TSH) within the anterior pituitary gland where it regulates the gene expression of cell type-specific markers, such as growth hormone and prolactin. Pit-1 belongs to the larger family of POU-domain DNA binding proteins that are characterized by a unique bi-partite DNA-binding motif consisting of two closely related protein segments that share homology with the superfamily of homeobox regulatory genes. Our knowledge of Pit-1 and the naturally occurring Pit-1 genetic mutations has provided one of the best model systems to study the role of POU-homeo domain genes in mammalian development - specifically, pituitary organogenesis. Structure-function studies suggest that the entire POU domain is required for correct recognition of the target genes activated by Pit-1. Indeed, mutations within either of the two domains, the POU-specific or POU-homeodomain, of this bi-partite motif results in a phenotype with clear pituitary dysfunction. Previous studies have shown that mutant mice or humans carrying a single mutation in the conserved DNA binding motif of Pit-1 display the loss of a subset of pituitary cell types expressing Pit-1 [3-5]. As expected, both rodent and human mutants exhibit combined deficiencies of all three hormones, GH, PRL and TSH. We have also identified a mutation in the Pit-1 gene from patients exhibiting both inherited short stature and combined deficiencies in GH, PRL and TSH. This mutant Pit-1 protein is unable to activate growth hormone and prolactin gene expression. Interestingly, loss of specific cell types does not seem to occur in these individuals, as evidenced by normal pituitary size [6]. The results of these genetic studies suggest that different segments of the Pit-1 protein mediate distinct events in pituitary development. These naturally occurring genetic variants will help us to understand how a single developmental regulator is able to specify multiple cell types. We wish to identify other tissue-specific co-activators that interact with Pit-1 to limit the expression and production of GH by somatotropes and PRL by lactotropes. Both biochemical and genetic approaches are being used to identify such co-activators. Our understanding of the precise molecular mechanisms that help Pit-1 to establish final differentiated cell types in the pituitary is part of the broader question of how cell-lineage and cell differentiation are determined. Such co-activators may contribute to the pituitary pathogenesis of GH- and PRL- secreting adenomas or other forms of pituitary-hormone deficiency not linked to mutations in the Pit-1 gene.

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Signal Transduction

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G PROTEINS IN TRANSMEMBRANE SIGNALING. H. R. Bourne, Departments of Pharmacology and Medicine, University of California, San Francisco, CA 94143-0450, USA

The heterotrimeric G proteins transduce signals from hormone receptors to regulate effector molecules — enzymes and ion channels — which in turn generate intracellular messengers that alter cellular function. Each member of the growing G protein family participates in a cycle of GTP binding and hydrolysis that amplifies hormonal signals. Mutations that interfere with this cycle cause or contribute to the pathogenesis of several endocrine diseases: 1. Pseudohypoparathyroidism, type I, in which patients inherit a defective copy of the α_s gene, which encodes the α subunit of G_s, the stimulatory regulator of adenylylcyclase. 2. Acromegaly, due in ~40% of cases to somatic mutations in pituitary somatotrophs that constitutively activate α_s and elevate cyclic AMP. 3. Tumors of thyroid, adrenal cortex, and ovarian granulosa cells, in which somatic mutations activate α_s or the α subunit of a different G protein, G_{i2}. 4. McCune-Albright Syndrome, in which an activating α_s mutation occurs in the early embryo, and eventually causes multiple endocrine tumors. In combination with the mutations found in these diseases, biochemical and molecular genetic analysis of α subunits have opened avenues to molecular understanding of the GTPase cycle, the 3-dimensional structure of G proteins, and signaling interactions of these proteins with receptors and effectors.

Sex Determination

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THE GENETICS AND BIOCHEMISTRY OF SEX DETERMINATION.
P. N. Goodfellow.

The Y chromosome is a dominant induced of testis development mammals. The identification and cloning of *SRY*, the testis determining gene, depended on the analysis of the genomes of patients with sex reversal syndromes. By analysis of Y chromosome fragments in the genomes of XX males, it was possible to define a 35kb minimum sex determining region(1); *SRY* (*sex determining region y gene*, the equivalent mouse gene is *Sry*) is located within this region(2). *De novo* mutations in *SRY* have been found in 15% of XY females(3,4,5) and XX mice transgenic for *SRY* are sex reversal males(6). The protein encoded by *SRY/Sry* contains an HMG box, a protein motif associated with DNA binding activity. Recent experiments have demonstrated DNA-binding activity of *SRY* protein and associated this biochemical activity with sex determination(7,8). This implies that the genes immediately "downstream" of *SRY/Sry* in the sex determination pathway will be subject to direct transcriptional regulation. Clues to the identity of these "downstream" genes may come from the analysis of the gnomes of Y-sequence negative XX males. Both "upstream" and "downstream" genes may be affected in XY females with intact *SRY* genes.

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Early Development

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DIFFUSIBLE FACTORS AND CELL DIFFERENTIATION.

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All adult organs consist of several different cell-types; these first appear in embryos, and are then maintained, and usually replenished from stem cells, in later life. In humans and in vertebrate animals, interactions between cells are of overwhelming importance in initiating (and maintaining) cell differentiation. Major advances have recently been made in identifying the kinds of molecules which can initiate cell differentiation in embryos through cell interactions. These include a number of known "growth factors" such as basic FGF, TGFβ, Activin, Wnt-8, etc.

These factors, which are products of genes active in early development, are usually short-lived and, in contrast to hormones, are effective only over short distances of a few hundred microns or less than 10 cell diameters. Special mechanisms regulate the distance, and hence numbers of cells, over which these factors exert an influence in early development. One of these is the limited time over which cells are competent to respond to signalling molecules. Another is the nature of the extracellular material by which cells adhere to each other.

The complete specialization of a cell-type usually depends on a sequence of cell interactions, such that cells formed as a result of one interaction, themselves emit a signal to which other cells respond.

Most of the known cell interactions are of the paracrine type involving an interaction between different kinds of cells. But an example is also known in which cells of like type interact with each other, by a "community effect", and apparently by an autocrine mechanism. The general principle by which cell differences are generated in early vertebrate development differs from a typical hormone action in two major respects. First the signals are strictly limited in the distance they travel from their source. Second, many more cells have the ability to respond to the signal (by possession of appropriate receptors) than actually respond in normal life.

Most of the locally acting diffusible factors of the kind just referred to have dramatically divergent effects on different cell-types at different stages of development. This diversity of response to the same factor may be achieved at the level of second messenger pathways or by the selective interaction of proteins with the promoters of early response genes. Examples of this last kind will be described.

Short-lived locally acting factors seem to be of widespread importance in adult stem cells as well as in embryos, and may therefore be of clinical importance in regulating the production of differentiated cells from stem cells, and in preventing the proliferation and spread of cancer cells.

Andrea Prader Lecture

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The Andrea Prader lecturer and awardee, selected by the European Society for Paediatric Endocrinology, will be announced at the meeting.