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PROCEEDINGS

THE TWENTY-EIGHTH MIDWINTER CONFERENCE OF IMMUNOLOGISTS
January 28-31, 1989
Asilomar Conference Center
800 Asilomar Avenue, Pacific Grove, California

TITLE: LYMPHOCYTE DIFFERENTIATION

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

SESSION I: DEVELOPMENT OF THE NERVOUS SYSTEM
Chairperson: Louis Reichardt

Speakers:

Louis Reichardt
University of California, San Francisco, CA
"Neuronal Glycoproteins that Regulate Axon Extension"

Martin Raff
University of London, London, UK
"Cell Diversification in the Mammalian Central Nervous Sytem"

Yuh Nung Jan
University of California, San Francisco, CA
"Genes Affecting Neurogenesis of Neuronal Identity in Drosophila"

Corey Goodman
University of California, Berkeley, CA
"Molecular Genetics of Neuronal Recognition and Growth Cone Guidance in Insects"

SESSION II EARLY EVENTS IN LYMPHOCYTE DIFFERENTIATION
Chairperson: Owen N. Witte

Speakers:

OWEN N. WITTE
University of California, Los Angeles, CA
"Oncogene Interactions with Primitive Lymphoid Cells"

DAVID BALTIMORE
The Whitehead Institute, Cambridge, MA
"Control of Immunodifferentiation: Inducing Rearrangement and Gene Expression"

DAVID WEAVER
Harvard University, Boston, MA
Gene Rearrangement and the Scid Mutation"

WILLIAM PAUL
National Institutes of Health, Bethesda, MD
"Lymphokines: Regulation of Immunoglobulin Class Expression and Production by Mast Cell Lines"

SESSION III CELLULAR INTERACTIONS IN LYMPHOCYTE DEVELOPMENT
Chairperson: ADA KRUISBEEK

Speakers:

ADA KRUISBEEK
National Institutes of Health, Bethesda, MD
"Positive Selection in Thymocyte Differentiation"

BARTON HAYNES
Duke University, Durham, NC
"Ontogeny of CD7+ T Cell Precursors: A Model for the Initial Stages of Human T Cell Development"

JONATHAN SPRENT
Scripps Clinic and Research Foundation, La Jolla, CA
"Tolerance in the Thymus"

CHRISTOPHER GOODNOW
University of Sydney, Australia
"Mechanisms of B Cell Tolerance"

SESSION IV ANTIGEN RECEPTOR EXPRESSION DURING THYMIC DIFFERENTIATION
Chairperson: James Allison

Speakers

JAMES ALLISON
University of California, Berkeley, CA
"Expression of $\alpha\beta$ and $\gamma\delta$ TCR During Thymic Differentiation"

MAX COOPER
University of Alabama, Birmingham, AL
"T Cell Development in Birds"

DENNIS LOH
Howard Hughes Medical Institute, St. Louis, MO
"T Cell Development: A Transgenic Model"

NICOLAS CRISPE
National Institute for Medical Research, Mill Hill, UK
"Genetic Control of the T Cell Receptor Repertoire"

SESSION V DEVELOPMENT OF B AND T CELL REPERTOIRE
Chairperson: Philippa Marrack

Speakers:

PHILIPPA MARRACK
National Jewish Hospital, Denver, CO
"The T Cell Repertoire Selection in the Thymus"

PAWEL KISIELOW
Basel Institute for Immunology, Basel, Switzerland
"T Cell Repertoire Selection in the Thymus"

MARTIN WEIGERT
Institute for Cancer Research, Philadelphia, PA
"Genetic Control of Autoantibodies"

ALAN STALL
Stanford University, Stanford, CA
"Repertoire Development of the Ly-1 B Cell Lineage"

LOUIS F. REICHARDT

Name

I

Session

Title: Neuronal Glycoproteins that Regulate Axon Extension

(Please include four references)

Neurons use a number of distinct glycoproteins that function as receptors to promote axonal extension on extracellular matrices and other cell types, such as Schwann cells, myotubes and astroglial cells. Neurons use different receptors to interact with different cell types. Different neurons also differ in the receptors that they utilize. The neuronal receptors and their ligands are regulated in ways capable of explaining major features of growth cone motility in vivo.

Bixby, J.L., Lilien, J., Reichardt, L.F. (1988) Identification of the major proteins that promote neuronal process outgrowth on Schwann cells in vitro. J. Cell Biol. 107: 353-361.

Neugebauer, K.M., Tomaselli, K.J., Lilien, J., and Reichardt, L.F. (1988) N-cadherin, NCAM, and integrins promote retinal neurite outgrowth on astrocytes in vitro. J. Cell Biol. 107: 1177-1187.

Tomaselli, K.J., Damsky, C.H. and Reichardt, L.F. (1988) Purification and characterization of mammalian integrins expressed by a rat neuronal cell line (PC12): Evidence that they function as α/β heterodimeric receptors for laminin and type IV collagen. J. Cell Biol. 107: 1241-1252.

Ignatius, M.J. and Reichardt, L.F. (1988) Identification and characterization of a neuronal laminin receptor: An integrin heterodimer that binds laminin in a divalent cation-dependent manner. Neuron 1: 713-725.

Martin Raff

Name

1

Session

Title: CELL DIVERSIFICATION IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

(Please include four references)

The rat optic nerve is one of the simplest parts of the CNS. It contains three types of macroglial cells - oligodendrocytes and two types of astrocytes: type-1 astrocytes form the glial limiting membrane at the periphery of the nerve and help form the blood-brain barrier, while type-2 astrocytes extend processes to nodes of Ranvier. In vitro studies suggest that the three types of macroglial cells arise by two distinct lineages: oligodendrocytes and type-2 astrocytes develop from a common, bipotential (O-2A) progenitor cell, whereas type-1 astrocytes develop from a different precursor cell. Type-1 astrocytes first appear at embryonic day 16 (E16), oligodendrocytes on the day of birth (E21), and type-2 astocytes around postnatal day 8-10 (P8-10). There is increasing evidence that O-2A progenitor cells migrate into the developing optic nerve from the brain.

What controls whether an O-2A progenitor cell develops into an oligodendrocyte or a type-2 astrocyte and why do oligodendrocytes first appear at birth while type-2 astrocytes only appear in the second postnatal week? In vitro experiments suggest that oligodendrocyte differentiation is the constitutive pathway of O-2A progenitor cell development, the timing of which is controlled by a clock in the progenitor cell that is driven by platelet-derived growth factor (PDGF) secreted by type-1 astrocytes. Type-2 astrocyte differentiation, on the other hand, seems to be induced by a 25,000 dalton protein that first appears in the developing optic nerve in the second postnatal week. There is evidence that this protein is ciliary neurotrophic factor (CNTF), which may also be secreted by type-1 astrocytes.

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Raff, M.C., Miller, R. and Noble, M. (1983) A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture media. Nature 308, 390-396.

Raff, M.C., Abney, E.R. and Fok-Seang, J. (1985) Reconstitution of a developmental clock in vitro: a critical role for astrocytes in the timing of oligodendrocyte differentiation. Cell 42, 61-69.

Raff, M.C. et al. (1988) Astrocyte-derived PDGF observes the clock that times oligodendrocyte development in culture. Nature 333, 562-565.

Hughes, S.M. et al. (1988) Ciliary neurotrophic factor (CNTF) induces type-2 astrocyte differentiation in culture. Nature 335, 70-73.

Yuh Nung Jan
Name

1
Session

Title: GENES AFFECTING NEUROGENESIS OR NEURONAL IDENTITY IN DROSOPHILA.

(Please include four references)

We are interested in early events of neural development. To study these events at the molecular level, we took a genetic approach and attempted to identify relevant genes. Several genes, such as Notch and scute, have been previously shown to affect neurogenesis in embryos. In order to look for more genes important for neural development we used the embryonic peripheral nervous system (PNS) as our assay system and examined embryonic lethal mutations and chromosomal deletions of possible defects. After screening approximately half of the Drosophila genome, we have found more than 20 regions or genes which when deleted or mutated, give rise to abnormal PNS. So far, we have been concentrating on two classes of mutations: (1) mutations affecting neurogenesis & (2) mutations affecting neuronal identity.

Several of these genes have been cloned by colleagues in our lab; including big brain (bib), neuralized (neu) daughterless (da) cut and numb. The possible biological significance of the molecular information will be discussed.

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Bodmer, R., Barbel, S., Shepard, S., Jack, J.W., Jan, L.Y. and Jan, Y.N. (1987) Transformation of sensory organs by mutations of the cut locus of D. melanogaster. Cell 51, 293-307.

Blochlinger, K.B., Bodmer, R., Jack, J., Jan, L.Y. and Jan, Y.N. (1988) Primary structure and expression of a product from cut locus involved in specifying sensory organ identity in Drosophila. Nature 333, 629-635

Caudy, M., Grell, E.H., Dambly-Chaudiere, C., Ghysen, A., Jan, L.Y. and Jan, Y.N. (1988) The maternal sex determination gene daughterless has zygotic activity necessary for the formation of peripheral neurons in Drosophila. Genes & Dev. 2, 843-852.

Caudy, M., Vaessin, H., Brand, M., Tuma, R., Jan, L.Y. and Jan, Y.N. (1988) daughterless, a gene essential for both neurogenesis and sex determination in Drosophila, has sequence similarities to myc and the achaete-scute complex. Cell, in press.

Corey S. Goodman
Name

I
Session

Title: Molecular Genetics of Neuronal Recognition and Growth Cone Guidance in Insects

(Please include four references)

We are interested in understanding the mechanisms underlying growth cone guidance and cell recognition during neuronal development. To address these issues, we use molecular genetic approaches to study the developing *Drosophila* and grasshopper embryos. Our cellular analysis gave rise to the labeled pathways hypothesis which predicts that bundles of axons (axon fascicles) in the developing embryo are differentially labeled by surface recognition molecules which help to guide growth cones toward their targets. Our molecular genetic approach has been three-fold. First, we have been studying the expression and function of cell and substrate adhesion molecules in *Drosophila* which are likely to play a significant role in these events. For example, we have cloned the three genes which encode the three subunits of *Drosophila* laminin, and are presently looking for mutations in these genes.

Second, we identified and cloned the genes for four different surface glycoproteins, called fasciclin I, fasciclin II, fasciclin III, and neuroglian, which are dynamically expressed on different overlapping subsets of axon fascicles and glia during development. Two of these molecules (fasciclin II and neuroglian) are part of the immunoglobulin superfamily and are highly related to a series of vertebrate neural cell adhesion molecules. The other two proteins (fasciclin I and III) are unrelated to anything else in the data bank. We have transvected the S2 cell line with the fasciclin III cDNA and have used cell aggregation assays to show that fasciclin III is a homophilic adhesion molecule which may define a new class of adhesion molecules. We are using genetic analysis and transformation methods to study the function of these molecules. Point mutations is both fasciclin I and fasciclin III are viable, but each mutant has striking behavioral abnormalities suggesting wiring defects in the CNS; the double mutant has more severe behavioral defects.

Third, we are using a variety of genetic methods to screen for new mutants which alter neuronal recognition. Based on our knowledge of the phenotypes of fasciclin I and fasciclin III single and double mutants, we are conducting mutant screens to look for similar and interacting genes.

Bastiani, M.J., Harrelson, A.L, Snow, P.M., and Goodman, C.S. (1987) Expression of fasciclin I and II glycoproteins on subsets of axon pathways during neuronal development in the grasshopper. Cell 48, 745-755.

Patel, N.H., Snow, P.M., and Goodman, C.S. (1987) Characterization and cloning of fasciclin III: a glycoprotein expressed on a subset of neurons and axon pathways in Drosophila. Cell 48, 975-988.

Zinn, K., McAllister, L., and Goodman, C.S. (1988) Sequence and expression of fasciclin I in grasshopper and Drosophila. Cell 53, 577-587.

Harrelson, A.L., and Goodman, C.S. (1988) Growth cone guidance in insects: fasciclin II is a member of the immunoglobulin superfamily. Science 242, 700-708.

Owen N. Witte, M.D.
Name

II
Session

Title: Oncogone Interactions with Primitive Lymphoid Cells

(Please include four references)

Activation of cellular proto-oncogenes as a result of chromosomal abnormalities has been implicated in the development of a number of human malignancies. Activation of the c-abl proto-oncogene is implicated in two forms of human leukemia: chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL). Over 95% of patients with CML and about 20% of patients with ALL have the karyotypic abnormalitiy known as the Philadelphia chromosome (Ph). This chromosomal aberration results from a reciprocal translocation between chromosomes 9 and 22 [t (9;22)(q34;q11)]. All but the first exon of the human c-abl gene is translocated within coding sequences of the breakpoint-cluster region (bcr) gene on chromosome 22. The fused bcr-abl gene gives rise to two alternative chimeric protein products of 210 Kilodaltons in CML and 185 Kilodaltons in ALL. The only known difference between the proteins is the absence of a segment of bcr sequences in $P185^{bcr\text{-}abl}$. As a consequence of amino-terminal structural alteration, both $P210^{bcr\text{-}abl}$ and $P185^{bcr\text{-}abl}$ have associated tyrosine kinase activities which are similar to that of the Abelson murine leukemia virus-encoded $P160^{gag\text{-}abl}$ and distinctive from the normal c-abl proto-oncogene. The P210, P185 and P160 proteins all function in the transformation of immature hematopoietic cells in several in vitro model systems. These data strongly support the role of the altered abl oncogene in Ph+ human leukemias and serve as a useful model to study growth regulation in pluripotent and lymphoid restricted progenitors.

McLaughlin, J., Chianese, E. and Witte, O.N. 1987. In vitro transformation of immature hematopoietic cells by the P210 bcr/abl oncogene product of the Philadelphia chromosome. Proc. Natl. Acad Sci., 84: 6558-6562.

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Rosenberg, N. and Witte, O.N. 1988. The viral and cellular forms of the Abelson (abl) oncogene. Advances in Virus Research 39:39-81.

Young, J.C. and Witte, O.N. Selective Transformation of Primitive Lymphoid Cells by the BCR/ABL Oncogene Expressed in Long Term Lymphoid or Myeloid Cultures. Mol. Cell. Biol. 8: 4079-4087.

DAVID BALTIMORE
Name

II
Session

Title: Control of immunodifferentiation: inducing rearrangement and gene expression

David Baltimore, David Schatz, Marjorie Oettinger, Patrick Baeuerle, Michael Lenardo, Jacqueline Pierce, Cornelius Murre and Patrick McCaw

Our interest in what triggers events of immunodifferentiation include studies in three areas. One is how the program is set in motion. The earliest events include steps of gene rearrangement. We have found that a relatively small piece of DNA can confer on a "fibroblast" (actually probably a mesenchymal stem cell) the ability to rearrange immunoglobulin genes. We have tagged the DNA with an oligonucleotide marker and are in the process of cloning it.

We have also been studying the proteins that regulate immunoglobulin gene expression. We will discuss how 2 of these--NK-κB and a protein binding to the kE2 motif--turn out to have much wider roles in biology. NF-κB, which is regulated by induction from a cytoplasmic pool of the protein bound to a specific inhibitor, is involved in many induction systems: T-cell activation and induction of β-interferon are 2 examples. The kE2-binding protein is a close homologue of the the Drosophila daughterless protein and a relative of myc, myoD and other control proteins. It is wide-spread in its expression and is likely to be involved in many gene expression systems. How such proteins come together to allow expression of immunoglobulin genes will be discussed.

David Weaver

Name

II

Session

Title: Gene Rearrangement and the scid mutation

(Please include four references)

The mouse *scid* (severe combined immunodeficiency) mutation blocks the differentiation of two cell lineages: B and T cells. We and others have shown that the *scid* mutation is associated with a gene rearrangement defect for both immunoglobulin (Ig) and T cell receptor (TCR) gene families. Endogenous Ig gene rearrangement is blocked at the D to Jh joining stage in every pre-B cell line that we have investigated; there is no evidence for V to DJh joining or light chain rearrangement. DNA sequencing and Southern blot analysis of cloned breakpoint junctions from the *scid* pre-B cells indicate that all the aberrant recombination events occur between the D and Jh regions of the Ig heavy chain chromosome, the normal first step in Ig gene rearrangement (Hendrickson et al., 1988). Therefore, our data indicates that *scid* cells have the normal timing and chromosomal gene region selection, but that the mutation may be focused on another function in gene rearrangement. There is no evidence of signal elements or pseudo-signal sequences at the novel *scid* breakpoint junctions that may have catalyzed gene rearrangement at their flanking sites. Double-strand breaks introduced randomly by XRays are as efficiently repaired in *scid* and wildtype pre-B cells, thus the *scid* mutation is probably specific to Ig gene recombination, rather than any other recombination event.

We have introduced into *scid* and wildtype pre-B cells two types of recombinant retroviral vectors containing rearrangement cassettes. In wildtype cells, the DGR cassette makes inversional rearrangements and the JVD cassette makes deletional rearrangements. In *scid* cells, the DGR and JVD rearrangements are aberrant, just as occurs with the endogenous Ig genes. This result demonstrates that the *scid* cells are active for continuing gene rearrangements in culture and importantly that the *scid* gene encodes a trans-acting factor critical for gene rearrangement. By a detailed inspection of these retroviral rearrangements, four types of events have been observed: 1)large deletions removing most of the retroviral DNA sequences, 2)small deletions centered over one heptamer, 3)hybrid joints formed between the signal heptamer of one partner and the coding sequences of the other, and 4)inversions accompanied by deletions. When inversions are selected for in DGR, the coding joints are frequently aberrant whereas the reciprocal or signal heptamer joint from the same rearrangements are normal. Several of these inversions are indistinguishable from normal rearrangements. Therefore, the *scid* defect is permissive for normal gene rearrangements at low levels. Point mutations introduced into one of the signal heptamers in JVD destroys rearrangement in wildtype pre-B cells. Interestingly, in *scid* cells, single point mutations do not significantly disrupt the generation of rearrangements, all of which are aberrant. Taken together, these results suggest that there are two phases in Ig gene rearrangements: site recognition/cleavage and rejoining of the rearranged double-strands. We have shown that the *scid* defect acts at the rejoining of the strands, particularly at coding joints. Thus, the inability to rejoin coding joints in endogenous Ig gene rearrangements as a result of the mutation directly correlates with the lack of Ig protein and lymphoid cell differentiation in *scid* mice.

Hendrickson,E., Schatz,D. and Weaver,D. (1988) The *scid* gene encodes a trans-acting factor that mediates the rejoining event of Ig gene rearranngement. Genes and Develpment 2: 817-829.

WILLIAM E. PAUL
Name

II
Session

Title: LYMPHOKINES: Regulation of Immunoglobulin Class Expression and Production by Mast Cell Lines

(Please include four references)

Distinct classes of immunoglobulins mediate specific biologic functions. Thus, the control of immunoglobulin class expression is a central issue in immunologic regulation. Although it had been recognized for some time that T cells played a role in determining immunoglobulin class expression, progress in this field has only come with the recognition that lymphokines have potent class specific regulatory activities. In particular, interleukin-4 (IL-4) causes striking induction of IgG1 and IgE production in B cells stimulated with lipopolysaccharide or with specific T cells while interferon gamma promotes the production of IgG2a. These lymphokines mediate their action prior to the expression of the specific isotype on the cell surface, suggesting they act prior to rather than after the switch event. In addition, IL-4 causes co-expression of IgG1 and IgE on the surface of a substantial number of cells and a purified IgG1, IgE-bearing cell population can be stimulated to secrete IgG1 and IgE, although individual cells secrete Ig of only a single isotype. These results suggest that a genetic event that does not require constant region gene deletion plays a role in the switching process. Administration of monoclonal anti-IL-4 antibody blocks IgE production but not IgG1 production in primary responses to Nippostrongylus infection and to treatment with anti-IgD. Perhaps more importantly, anti-IL-4 strikingly inhibits specific secondary IgE responses and partially inhibits IgE responses to reinfection with Nippostrongylus. These results raise the possibility that interfering with the action of IL-4 may be useful in the therapy of allergic disorders.

We recently reported that many transformed murine mast cell lines secrete IL-4. We have now established that factor-dependent, non-transformed mast cell lines can be stimulated by ionomycin or by cross-linkage of high affinity receptors for IgE to secrete or to express mRNA for IL-3, IL-4, IL-5 and IL-6 but not IL-2, interferon gamma or lymphotoxin. This pattern of lymphokine release is similar to that of Th2 helper T cells. These results strongly suggest that mast cells may be an alternative source of lymphokines and that, in addition to their role as effectors, they may play an important regulatory role in allergic inflammation.

Ada M. Kruisbeek — III

Name — *Session*

Title: Positive Selection in Thymocyte Differentiation

(Please include four references)

The central theme of our research has been to analyze the development of the T cell repertoire under conditions where expression of particular MHC-encoded gene products is blocked by in vivo anti-MHC mAb treatments. Initial studies (1,2) demonstrated that generation of $CD4^+CD8^-$ cells was abrogated in mice treated from birth with anti-class II- mAb, establishing that the majority of $CD4^+CD8^-$ T cells require interactions with class II - MHC for their development to occur. Similar requirements apply to the development of $CD4^-CD8^+$ T cells: blocking of class I- MHC glycoproteins with anti-class I - mAb results in failure to generate $CD4^-CD8^+$ T cells (3). TCR-alpha beta repertoire analysis (through staining with V beta-chain specific antibodies) is currently performed on mice is which particular class I - MHC- (K-region or D-region encoded)- or class II -MHC (I-A or I-E)- gene products are blocked by specific mAb treatment during early development. The first such approach yielding clearcut results demonstrates that development of V beta 17^+ $CD4^-CD8^+$ T cells in the SJL ($H\text{-}2^s$) mouse strain is selectively abrogated by blocking of class $I\text{-}K^s$ molecules, but is not affected by blocking of class $I\text{-}D^s$ molecules (4). These data directly demonstrate that generation of $CD4^-CD8^+$ T cells expressing a particular TCR V-beta segment can be correlated with expression of a particular class I-MHC molecule, and thus provide evidence for positive selection. Our results thus confirm the suggestions for thymic selection derived 10 years ago from chimera models. Together with the recent demonstration that in yet another model, that of alpha-beta- TCR transgenic mice, positive selection in the context of thymic MHC occurs, it now appears that a firm place for a role of TCR-MHC interactions in the development of T cells has been established. Future studies should focus on how these models can be applied to formulate the mechanisms responsible for selection of TCR- specificities. In additional studies, we explored the role of the CD4 accessory molecules in the selection process. In vivo and in vitro blocking with anti-CD4 mAb's incapable of directly depleting $CD4^+CD8^-$ cells (i.e., Fab or $F(ab')_2$ fragments of GK1.5) during early development was applied. Under such conditions (i.e., blocking of the CD4 molecules), a selective failure to develop $CD4^+CD8^-$ T cells was observed, documenting that the CD4 molecules play an active role in positive selection. Again, resolving the mechanisms responsible for this participation is the subject for future analysis.

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3. Marusic-Galesic, Stephany, Longo, and Kruisbeek, Nature 333: 180-183, 1988.

4. Zuniga-Pflucker, Longo, and Kruisbeek, Nature, in press, 1989.

Barton F. Haynes, M.D.

Name

III

Session

Title: ONTOGENY OF CD7+ T CELL PRECURSORS: A MODEL FOR THE INITIAL STAGES OF HUMAN T CELL DEVELOPMENT, Barton F. Haynes, Stephen M. Denning, Kay H. Singer and Joanne Kurtzberg, Departments of Medicine and Pediatrics, Duke University Medical Center, Durham, North Carolina 27710

(Please include four references)

Understanding the earliest stages of T cell development is important for study of immunodeficiency diseases and for developing effective methods of immune reconstitution. In addition, understanding the genesis of autoimmunity necessitates knowing how cells of the thymic microenvironment interact with immature T cell precursors. We have used monoclonal antibodies that react with T cell precursors prior to their entry into the thymic rudiment, coupled with progenitor assays to define and characterize cells capable of differentiation to the T lineage. Moreover, we have established in vitro assays of thymocyte-thymic epithelial (TE) cell interactions. We have used the CD7 monoclonal antibody to isolate CD7+ cells from first trimester fetal tissues and postnatal thymus, and as well to study CD7+, CD4-, CD8-, CD3- (triple negative) leukemic cells. CD7+ triple negative normal and malignant cells upon stimulation with PHA-conditioned media and IL2 gave rise to mature T cells. Interestingly, both normal and leukemic CD7+ triple negative cells, when cultured under conditions favorable for myeloid differentiation, gave rise to CFU-GEMM and CFU-GM colonies as well. The presence of 1,14 or 7,14 chromosome translocations in two of the CD7+ leukemic suspensions allowed for a precursor-product relationship to be determined, demonstrating that leukemic CD7+ triple negative cells were indeed multipotent. These data have given rise to a model pathway of early T cell maturation whereby CD7+ multipotent T cell precursors (pro-T cells) colonize the human thymus and give rise to committed pre-T cells which in turn give rise to T cells expressing either $\alpha\beta$ or $\gamma\delta$ T cell receptors. Once in the thymic microenvironment, immature T cells express surface CD2 and CD18 (LFA-1) molecules and bind to TE cells. Thymocyte CD2 molecules interact with TE LFA-3 molecules, and thymocyte CD18 molecules interact with TE cell intercellular adhesion molecule-1 (ICAM-1) molecules. Activated TE cells produce a number of growth and differentiation cytokines (IL-1, G-CSF, M-CSF, and GM-CSF) that are capable of mediating a variety of actions on intrathymic T cell precursors. Taken together, these data suggest pathways of early T cell maturation, and define cytokines and surface molecules of TE cells that may play important roles in the early stages of T cell precursor proliferation and differentiation.

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Jonathan Sprent
Name

III
Session

Title: Tolerance in the thymus

(Please include four references)

T cell tolerance to H-2 determinants was investigated in parent → F_1 chimeras given supralethal irradiation. Despite preparing the chimeras with up to 2300 rad (given in divided doses) the donor-derived $CD4^+$ cells developing in the chimeras showed substantial tolerance to the host in terms of primary MLR and complete tolerance for induction of lethal graft-versus-host disease. In $I\text{-}E^-$ → $I\text{-}E^+$ combinations, tolerance of $CD4^+$ cells was associated with ≃ 75% deletion of $V_\beta 11^+$ cells. Tolerance did not appear to occur post-thymically because tolerance was not seen in thymectomized irradiated (a x b)F_1 hosts given parent a marrow plus a parent a neonatal thymus graft. Evidence for tolerance occurring within the thymus itself came from the finding that mature thymocytes from long-term parent → F_1 chimeras showed near-complete tolerance to host-type H-2 determinants. Collectively the data favor the view that tolerance of $CD4^+$ cells is controlled, at least in part, by a component of thymic epithelium.

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Christopher C. Goodnow
Name

III
Session

Title: Mechanisms of B Cell Tolerance

(Please include four references)

The phenomenon of self-tolerance involves two key issues: what variables influence whether an autologous antigen is recognised as "self", and what is the fate of self-reactive cells following self-recognition. To address these issues, two types of transgenic mice have been produced. The first type carries one of two gene constructs encoding a "neo-self" antigen, hen egg lysozyme (HEL). Expression of the HEL transgene is driven by a heterologous promoter, derived from either the mouse metallothionein or mouse albumin genes. Multiple lines of HEL transgenic mice have been produced on a C57BL/6 inbred background, and through the combination of position effects, different promoters, and zinc induction it is possible to compare tolerance to HEL in groups of mice expressing a range of levels of serum HEL, from less than 0.2 ng/ml to 100 ng/ml. Tolerance to HEL is manifest in all lines with detectable basal levels of HEL.

The second type of transgenic mouse carries rearranged immunoglobulin heavy (μ-δ) and light chain genes encoding a high affinity anti-HEL antibody. Greater than 90% of the B-cells in these mice express only the transgene-encoded anti-HEL antibody as cell-surface IgM and IgD. These B-cells are localised primarily in the follicular areas of lymph node and in both the follicular and marginal zones of the spleen. Six lines of μ-δ + κ Ig-transgenic mice have been characterised, and mated to various HEL-transgenic lines, to produce "double-transgenic" mice carrying both the HEL transgene and the antibody transgenes. Secretion of anti-HEL antibody is curtailed in most combinations of HEL-transgenic and Ig-transgenic lines, accompanied by a marked and selective reduction in the levels of surface IgM but no change in IgD. Intriguingly, HEL-binding B-cells are depleted from the marginal zones but not from the follicles in double-transgenic spleen. By contrast, in double-transgenic mice expressing 20-fold lower levels of HEL, secretion of anti-HEL antibody continues to a significant extent and there is only a slight reduction in surface IgM on the B-cells. B-cell tolerance in this model therefore appears to be antigen-dose dependent and intimately linked to surface IgM downregulation.

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James P. Allison — *Name*

IV — *Session*

Title: Expression of αβ and γδ TCR during thymic differentiation

(Please include four references)

The vast majority of T cells in the lymphoid organs of mice express antigen receptors (TCR) composed of CD3-associated αβ heterodimers, while a minor fraction express CD3-associated γδ heterodimers. According to the conventional view, both types of TCR are generated by random rearrangements of elements of the TCR loci during intrathymic differentiation. Contributions from the different V regions available in the germline, from combinatorial joining of V, D, and J segments, and from N-region diversification give rise to an enormously diverse population of TCR molecules from which the functional T cell repertoire is selected. Our studies have shown that this diversity is not manifested in at least one tissue, the epidermis. Molecular and serologic analysis of Thy-1+ dendritic epidermal cells (dEC) from adult mice has revealed that a very restricted repertoire derived solely from Vγ3-Jγ1 and Vδ1-Dδ2-Jδ2 rearrangements with essentially no N-region diversity, properties suggestive of a fetal origin of the dEC. We have found that during fetal thymic ontogeny three waves of cells expressing γδ TCR, and a single wave of cells bearing αβ TCR, emerge successively. The first of these is composed of cells which, like adult dEC, express Vγ3, suggesting that the fate of these first thymic emigrants is to seed the epidermis, while subsequent waves of cells seed other peripheral tissues. Thus it appears that the development of different types of T cells is highly regulated, and that the timing of the highly ordered rearrangement of TCR gene segments has a profound effect on the emergence and ultimate tissue distribution of T cells. It also appears that thymocyte differentiation may be divided into two broad stages according to the availability of the complete machinery of rearrangement. The earliest stage involves generation of cells bearing specific Vγ and Vδ gene segments with restricted diversity which may directly and without selection seed the epithelial tissues. In the second stage, additional Vγ and Vδ, as well as the complete complement of Vα and Vβ genes are available for use and the complete rearrangement machinery is operative, allowing generation of genes with the full extent of junctional diversity and selection of the functional repertoire.

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Max D. Cooper, M.D.
Name

IV
Session

Title: T Cell Development in Birds

(Please include four references)

The avian thymus is colonized by serial waves of hemopoietic stem cells, the first of which enters the chick embryo thymus around day seven (E7). Within the thymus, these precursor cells give rise to three successive wavelets of T cells. These secondary waves reflect the sequential development of T cell sublines, each of which appears to express a different type of T cell receptor. Monoclonal antibodies have been used to characterize the different TCR isotypes and the T cells that express them. TCR1 cells express an avian homologue of the mammalian $\gamma\delta$/CD3 receptor complex. Their intrathymic development begins on E12. TCR2 cells, which express $\alpha\beta$ receptors, begin to appear on E15. TCR3 cells, which appear to express a novel TCR isotype, appear on E18. Migration of TCR1, TCR2 and TCR3 cells to the periphery occurs in this same order, and the tissue homing patterns for the T cell subpopulations are distinctive. This developmental process is repeated multiple times to generate a long-lived population of T cells that are clonally and isotypically diverse. Avian and mammalian T cell development will be compared in this presentation, the focus of which will be the TCR3 cell.

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Dennis Y. Loh, M.D.
Name

IV
Session

Title: T cell development: A transgenic model

(Please include four references)

Two of the interesting and fundamental processes during T cell development are the acquisition of MHC restriction and self-tolerance. We have tried to develop a model to study these phenomena through the use of transgenic mice bearing either (a) self-reactive/alloreactive T cell receptor or (b) Class II MHC antigens expressed in specific targeted tissues. By following the fate of the transgenic T cell receptor molecule in different H-2 environment, we have been able to elucidate positive and negative selection working on the developing T lymphocytes. These processes, we believe, are the origin of MHC restriction and self-tolerance to antigens present in the thymus.

To study the mechanism of tolerance development to peripheral antigens, we have created a transgenic mouse that expresses an MHC class antigen exclusively in the exocrine pancreas. The transgene is expressed but the pancreas is tolerated even in a potentially allo-reactive environment. Studies are underway to elucidate the mechanism of peripheral tolerance induction.

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I.N.CRISPE

Name

IV

Session

Title: GENETIC CONTROL OF THE T CELL RECEPTOR REPERTOIRE

(Please include four references)

Antigen receptors of the alpha-beta type are expressed at two distinct levels during differentiation in the thymus. Low level expression is seen in the fetus, and on CD4+,CD8+ cortical-type cells in the adult (1,2). Higher level expression is seen first in the neonate, and also occurs on medullary-type cells in the adult. During differentiation, the pattern of T cell receptor V region expression is shaped by the deletion of self-reactive cells (3), and by the positive selection of T cell receptors potentially restricted to self-MHC. The exact stage at which these selective events occur is not defined, although it is probably after the expression of CD4 and CD8 since those CD4-,CD8- cells which express alpha-beta antigen receptors do not act as precursor cells, and are probably a sterile offshoot of the main differentiation pathway (4).

Positive selection requires an interaction between T cell receptors and thymic MHC molecules, which is possibly analogous to restricted recognition in the periphery. The molecular mechanics of this interaction are unknown; in particular, the presence or absence and the functional significance of peptides in association with thymic MHC is undefined. Studies will be described in which non-MHC genes exert a major effect on positive selection, arguing that polymorphic self-components are recognised during this process.

Structural information about the HLA-A2 molecule plus extrapolations from immunoglobulin to the T cell receptor combining site have led to the suggestion that these two structures have intrinsic affinity, and may have co-evolved. The remarkable evolutionary conservation of both T cell receptor V regions and MHC loci between mouse and man supports this idea. If this is true, could particular Vregions have evolved in tandem with particular MHC molecules Data will be presented to suggest that one V region can be positively selected by either class I or class II MHC. Perhaps class I and class II tertiary structures are constrained to very close similarity by co-evolution with T cell receptor V regions.

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Philippa Marrack

Name

V

Session

Title: The T Cell Repertoire

(Please include four references)

Most T cells use clonally variable αβ receptors on their surfaces to recognize antigenic fragments bound to major histocompatibility complex proteins on cells. In most cases it seems that all the variable components of αβ receptors contribute to binding of the receptor to its ligand. Thus, VβDβJβ, VαJα and N regions all appear to play a role in the interaction.

Recently, we have come across some unexpected exceptions to this role. In these cases a particular antigen/MHC complex appears to bind well to Vβ. binding to the other variable components of the receptor does not seem to be important. Antigen/MHC complexes of this type stimulate large numbers of T cells, since they stimulate all T cells bearing the appropriate Vβ regardless of the make-up of the rest of the receptor on such cells. Because of these stimulatory effects we have termed such ligands "super antigens. These findings document a novel mechanism for T cells to recognize certain ligands.

Super antigens are probably of practical interest since they include proteins encoded in the mouse genome (Mls) and some bacterial enterotoxins.

These antigens can be used to follow the fate of potentially responsive T cells in immunized or suppressed animals.

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Pawel KISIELOW

Name

V

Session

Title: T Cell Repertoire Selection in the Thymus

(Please include four references)

To examine selection mechanisms involved in forming a mature (i.e. self MHC-restricted, self tolerant) T cell repertoire, T-cell-receptor (TCR) transgenic mice were constructed, which expressed on a large fraction of their T cells an $\alpha\beta$ TCR specfic for the male (H-Y) antigen in the context of class I H $2D^b$ MHC molecules.[1] Negative selection was analysed in make transgenic mice, whereas positive selection was analysed in female transgenic mice. The results indicate that interactions of $\alpha\beta$ TCR expressed on $CD4^+8^+$ thymocytes with ligands presented in the thymus have the following consequences:

- Binding to specfic self antigen presented by thymic MHC molecules leads to clonal deletion[2]
- Binding to polymorphic portions of restricting MHC molecules in the absence of specific nominal antigen leads to positive selection[3]
- Binding to class I MHC molecules induces the differentiation towards $CD4^-8^+$ T cells, whereas binding to class II MHC molecules induces the differentiation towards $CD4^+8^-$ T cells[4].

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Dr. Alan M. Stall | V

Name | *Session*

Title: Repertoire Development in the Ly-1 B Cell Lineage

(Please include four references)

Ly-1 B cells constitute a separate developmental B cell lineage. They are distinguished from the conventional B cell lineage with respect to their cell surface phenotype, functional repertoire and ontogeny (1). In contrast to the conventional B lineage which is continuously replenished from unrearranged Ig^- progenitors throughout the life of the animal, such progenitors give rise to Ly-1 lineage B cells only during fetal and neonatal life. Adoptive transfer studies show that from birth, bone marrow-derived Ig^- progenitors become increasingly incapable of generating new Ly-1 B cells, until, by 6-8 weeks there are essentially no new additions to the Ly-1 B cell compartment. In addition the mature Ig^+ Ly-1 B cell population exerts an inhibitory feedback on the development of Ly-1 B cells from Ig^- progenitors (2).

This unique ontogeny of Ly-1 B cells places severe restrictions on repertoire development within the lineage, in that, the repertoire develops early in B cell ontogeny and is fixed by 6-8 weeks of age in contrast to the conventional repertoire which is constantly renewed. In the absence of new V gene rearrangements, the adult Ly-1 B cell population would appear to be highly sensitive to alterations (mutations and/or deletions) in the repertoire. That this is in fact the case is indicated by studies at the cellular and molecular level which show that Ly-1 B cells have a limited functional repertoire which becomes increasingly restricted with age (3,4). This ongoing process inevitably leads to the development of oligoclonal (and eventually monoclonal) populations of Ly-1 B cells in elderly animals which often give rise to neoplastic populations similar to human B-chronic lymphocytic leukemia (3).

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