22. This compound was first prepared by R. Gradl and F. Lux. Reference to a synthesis can be found in the structure report: V. W. Day, T. J. Marks, W. A. Wachter, *J. Am. Chem. Soc.* **97**, 4519 (1975).
23. L. De Cola, D. L. Smailes, L. M. Vallarino, *Inorg. Chim. Acta* **110**, L1 (1985).
24. D. H. Busch and C. Cairns, in *Synthesis of Macrocycles: Progress in Macrocyclic Chemistry*, R. M. Izatt and J. J. Christensen, Eds. (Wiley, New York, 1987), vol. 3, pp. 1–51.
25. D. H. Busch *et al.*, *J. Am. Chem. Soc.* **103**, 1472 (1981).
26. N. Herron *et al.*, *ibid.* **105**, 6585 (1983).
27. N. Herron, J. H. Cameron, G. L. Neer, D. H. Busch, *ibid.*, p. 298.
28. P. Saltman and J. Hegenauer, Eds., *The Biochemistry and Physiology of Iron* (Elsevier Biomedical, Amsterdam, 1981).
29. B. F. Matzanke, G. Müller-Matzanke, K. N. Raymond, in *Physical and Bioinorganic Chemistry*, T. M. Loehr, H. B. Gray, A. B. P. Gray, Eds. (VCH, Deerfield Beach, FL, 1989), pp. 1–121; K. N. Raymond, G. Müller, B. F. Matzanke, *Top. Curr. Chem.* **123**, 50 (1984); J. B. Nielands, *Annu. Rev. Biochem.* **36**, 285 (1982).
30. M. E. Cass, T. M. Garrett, K. N. Raymond, *J. Am. Chem. Soc.* **111**, 1677 (1989).
31. F. L. Weitl and K. N. Raymond, *ibid.* **101**, 2728 (1979); M. C. Venuti, W. H. Rastetter, J. B. Nielands, *J. Med. Chem.* **22**, 123 (1979); F. L. Weitl, W. R. Harris, K. N. Raymond, *ibid.*, p. 1281.
32. S. J. Rodgers *et al.*, *Inorg. Chem.* **26**, 1622 (1987).
33. T. M. Garrett, P. W. Miller, K. N. Raymond, *ibid.* **28**, 128 (1989).
34. T. J. McMurry *et al.*, *J. Am. Chem. Soc.* **109**, 7196 (1987).
35. T. J. McMurry, S. J. Rodgers, K. N. Raymond, *ibid.*, p. 3451.
36. T. M. Garrett *et al.*, in preparation.
37. Crystal structures of the unsubstituted tris(catechol) ferric complexes show approximately octahedral coordination: K. N. Raymond, S. S. Isied, L. D. Brown, F. R. Fronczek, J. H. Nibert, *J. Am. Chem. Soc.* **98**, 1767 (1976); B. F. Andersen, D. A. Buckingham, G. B. Robertson, J. Webb, *Nature* **262**, 722 (1976).
38. F. Basolo and R. G. Pearson, *Mechanisms of Inorganic Reactions* (Wiley, New York, 1967).
39. Y. Sun, A. E. Martell, R. J. Motekaitis, *Inorg. Chem.* **25**, 4780 (1986).
40. C. J. Rodgers, C-Y. Ng, K. N. Raymond, *J. Am. Chem. Soc.* **107**, 4094 (1985).
41. C. O. Dietrich-Buchecker and J.-P. Sauvage, *Chem. Rev.* **87**, 795 (1987).
42. ———, J. M. Kern, *J. Am. Chem. Soc.* **106**, 3043 (1984). The first template catenane synthesis used a slightly less efficient stepwise route reported earlier by Sauvage *et al.* C. O. Dietrich-Buchecker and J.-P. Sauvage, *Tetrahedron* **24**, 5091 (1983) and references therein.
43. A.-M. Albrecht-Gary, C. O. Dietrich-Buchecker, Z. Staad, J.-P. Sauvage, J. Weiss, *Chem. Commun.* **1986**, 1325 (1986).
44. C. O. Dietrich-Buchecker, A. Khemiss, J.-P. Sauvage, *ibid.*, p. 1376.
45. J.-P. Sauvage and J. Weiss, *J. Am. Chem. Soc.* **107**, 6108 (1985).
46. We thank our past and present co-workers for their contribution to the work from this laboratory, which is referenced herein.

Developmental Biology of T Cell Receptors

JACK L. STROMINGER

T cell receptors are the antigen-recognizing elements found on the effector cells of the immune system. Two isotypes have been discovered, TCR-γδ and TCR-αβ, which appear in that order during ontogeny. The maturation of prothymocytes that colonize the thymic rudiment at defined gestational stages occurs principally within the thymus, although some evidence for extrathymic maturation also exists. The maturation process includes the rearrangement and expression of the T cell receptor genes. Determination of these mechanisms, the lineages of the cells, and the subsequent thymic selection that results in self-tolerance is the central problem in developmental immunology and is important for the understanding of autoimmune diseases.

THE CURRENT ERA OF STUDIES OF IMMUNE RECOGNITION began with the discoveries that both transplantation rejection and immune responsiveness were controlled by polymorphic cell surface molecules. A single genetic region of all vertebrate species examined, the major histocompatibility complex (MHC) (located on chromosome 6 in man and on chromosome 17 in the mouse), encodes two classes of cell surface molecules. The primary role of these molecules, the class I and class II MHC antigens, is the presentation of foreign peptides derived from foreign antigens to the effector cells of the immune system—that is, to the T lymphocytes. Thus, rejection of transplanted organs is a by-product of the essential role that the polymorphism of these molecules plays in immune recognition, ensuring that a large number of foreign peptides will be recognized by the species and ensuring sufficient population diversity that the species as a whole will escape potentially catastrophic variations in environmental agents—for example, viruses. In the past several decades, an enormous amount has been learned about the structure and the function of these two classes of molecules and about the genes that encode them (*1*). Moreover, it was shown that T lymphocytes recognize foreign antigens in an MHC-restricted fashion—that is, the effector T lymphocytes from one individual recognize a foreign antigen presented by cells of another individual only if the two individuals share at least one allelic MHC antigen (*2*). A long debate ensued as to whether the effector cell contained two receptors, one for foreign antigen and one for the MHC restricting element, or only a single receptor that recognized both. Little doubt remains now that a single receptor recognizes the complex of foreign peptide with MHC antigen (*3*).

For several years the nature of this receptor, called the T cell receptor (TCR), was elusive. The search for this molecule was based on the idea that the diversity needed to recognize a very large number of foreign antigens in association with many different MHC molecules would be generated by mechanisms analogous to those used in the generation of diversity of immunoglobulins. About 5 years ago such a molecule was identified, first as a heterodimeric protein on the surfaces of murine and human T cells identified by clone-specific monoclonal antibodies, and then as the α and β genes that encode the two chains of the protein. As had been predicted, these genes are encoded as V, J, and C segments of the α gene on chromosome 14 in both man and mouse, and as V, D, J, and C segments of the β gene on chromosome 7 in man and on chromosome 6 in the mouse. The diversity of the α and β genes is generated by the selection of different segments for joining and by

The author is a professor in the Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138, and at the Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

the insertion of random nucleotides (called N segments) between the rearranging VJ or VDJ elements. Somatic mutation, which is an important mechanism in the generation of diversity of immunoglobulin genes, does not appear to play a role in the generation of the diversity of TCR chains. This originally discovered T cell receptor is now called the αβ T cell receptor, or TCR-αβ (*4*).

In the course of the work on TCR-αβ, a third rearranging T cell gene also comprised of V, J, and C segments was discovered, first in the mouse (*5*) and then in man (*6*), and called TCR γ. T cell receptor gene rearrangements and most, if not all, of the process of T cell maturation occur in the thymus. During ontogeny, TCR γ gene rearrangements precede TCR α and β rearrangements (or TCR-αβ expression). TCR γ expression in the murine thymus reaches relatively high levels early in fetal development and then declines prior to birth. Subsequently, a second T cell receptor, TCR-γδ, and then the gene segments encoding the fourth chain of the rearranging T cell receptor family, TCR δ, were discovered (*7*). This second receptor is found on only 1% to 10% of peripheral blood T cells in man or the mouse, but in the chicken these T cells represent 30% (*8*). The function of this second T cell receptor remains enigmatic.

The development of the T cell repertoire within the thymus involves the rearrangement of the TCR α, β, γ, and δ genes and the formation of the TCR-γδ and TCR-αβ receptors. This process is accompanied by the selection for export to the periphery of those T cells that recognize foreign antigens in the context of an MHC molecule (positive selection) but which are not self-reactive (negative selection), at least in the case of cells bearing TCR-αβ. This review focuses on some issues in the development of TCRs raised by the discovery of TCR-γδ and by the fact that its appearance in ontogeny precedes that of TCR-αβ.

Three Waves of TCR Development

Three types of TCR-expressing cells are known: (i) $CD4^-CD8^-$ (double negative) TCR-γδ–expressing cells; (ii) $CD4^+CD8^+$, $CD4^+CD8^-$, or $CD4^-CD8^+$ TCR-αβ–expressing cells; and (iii) $CD4^-CD8^-$ TCR-αβ–expressing cells. These cells appear in the above order at different times in ontogeny. Thus, TCR-expressing cells appear in three distinct waves (*5, 8–14*). TCR-γδ and TCR-αβ are always associated on the cell surface with CD3, a complex of five polypeptides, CD3γ, δ, ε, ζ, and η, thought to be involved in signal transduction through the receptors.

The first TCR to appear in ontogeny is TCR-γδ. Most TCR-γδ cells are $CD4^-CD8^-$. However, a small percentage are clearly $CD8^+$, and $CD4^+$ TCR-γδ cells may also occur in even smaller numbers. The early discovery that the TCR γ gene was expressed at very high levels at 15 days of gestation in the mouse (*5, 9*), coupled with more recent similar information about the TCR δ gene (*7, 11*) and the TCR-γδ receptor (*8, 10*), prompted interest in the developmental appearance and role of this receptor. Rearrangements of Vγ in the murine thymus begin at day 14 and are complete by day 16 (Fig. 1). Transcription of TCR γ begins immediately, reaches a maximum by day 15, and thereafter declines until birth. Only a single 1.5-kilobase TCR γ transcript has been seen, and there is thus no evidence for transcription initiating immediately upstream of Jγ segments, which would yield a shorter transcript. Less is known about rearrangements of the V, D, and J segments of TCR δ, but the information that is available suggests that δ gene rearrangement and transcription are initiated in parallel with the formation of TCR γ. Further peculiarities of TCR γ transcription include the apparent preferential use of some Vγ, Jγ, and Cγ segments early in fetal development, as compared to the use of other segments in neonatal and adult life, and the shifting to a high incidence of out-of-frame

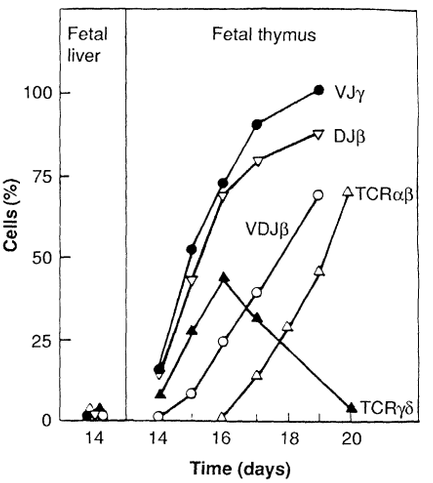

Fig. 1. TCR gene rearrangements and expression during fetal ontogeny in the mouse. This figure is adapted from (*12*) and other data (*5, 8–14*).

rearrangements by day 17. The surface expression of TCR-γδ begins at day 15, reaches a maximum by day 17, and thereafter declines until birth. The wave of TCR-γδ appearance may be further subdivided into as many as three waves, defined by preferential or sequential use of Vγ or Vδ segments; for example, in the mouse Vγ3 is used first and may be followed by a wave in which Vγ4 is used (*15*).

The second wave of TCR expression involves cells expressing TCR-αβ with CD4, CD8, or both. Rearrangement of DJβ begins at the same time as VJγ rearrangement at days 14 and 15 in the mouse, but VDJβ rearrangements are delayed until day 16. Correspondingly, at days 14 and 15, a 1.0-kb TCR β RNA transcript is seen (presumably initiating upstream from rearranged DJβ segments), and by day 16, a full-length 1.3-kb transcript is seen. The organization of the TCR α locus has made it difficult to observe TCR α gene rearrangements, but they are clearly delayed with respect to TCR β rearrangements, since TCR α transcription is first seen at day 16 in the form of a 1.4-kb short transcript, which presumably arises by initiation upstream from a Jα segment. The full-length 1.7-kb TCR α transcript does not appear until day 17 and is presumably correlated with VJα rearrangement. Because the entire δ-chain locus is situated in the middle of the TCR α locus (with Vα segments 5′ and Jα and Cα segments 3′), rearrangements of VJα are obligatorily coupled to deletion of the δ locus. Also at day 17, surface expression of TCR-αβ is initiated and reaches a maximum around the time of birth at 20 days. The relation of TCR-αβ expression to expression of CD4 and CD8, and the probable maturation of $CD4^+CD8^-$ and $CD4^-CD8^+$ TCR-αβ cells from $CD4^+CD8^+$ TCR-αβ precursors, are of considerable interest (*16*), but a detailed discussion is beyond the scope of this review. The nature of the TCR and whether its restricting element is a class I or class II MHC molecule determines, in a manner which is not understood, whether the T cell becomes $CD4^-CD8^+$ or $CD4^+CD8^-$, respectively. Similarly, the process of thymic selection, which results in the death of all but 1% of TCR-αβ–expressing cells and eliminates before export to the periphery all those that would recognize self, is a subject of major importance in immunology. Studies in several systems have focused on clonal deletion in response to self antigens as well as foreign antigens to which an individual is exposed neonatally (*17*). It is likely that this process is important in development of tolerance at the $CD4^+CD8^+$ stage of development within the thymic cortex.

Finally, the third type of cell expressing a T cell receptor is a $CD4^-CD8^-$ TCR-αβ–expressing cell (*18*). These cells appear late in development in the mouse, between birth and day 5 of life, and thus cannot be precursors of the $CD4^+$ or $CD8^+$ TCR-αβ–bearing cells that are already present at birth. They express low levels of

TCR-αβ and show a preferential use of the $V_\beta 8$ segment. Little is known about their function. The $CD4^-CD8^-$ TCR-αβ–expressing cells have only recently been found in human tissue (*19*). A third type of TCR appears just before hatching in the chicken (*20*); this TCR may represent a distinct third isotype of TCR or it may represent a TCR with unusual α and β gene segments. Although it appears at about the same time as the $CD4^-CD8^-$ TCR-αβ cells in the mouse, no data are available relative to the functional equivalence of these two types of cells. The embryonic chicken thymus is colonized intermittently in discrete temporal waves. These temporal waves do not, however, correspond to waves of different TCRs, because TCR-γδ and TCR-αβ cells appear at each temporal wave (*21*).

Most of these studies of TCR development were done in the mouse and the chicken. In general, the studies in human tissue have been limited by the difficulty of obtaining stage-specific human fetal thymocytes. Instead, reported studies have relied largely on material derived from leukemias and lymphomas, which have been staged by the phenotype of expressed surface antigens. They are therefore considerably less detailed than the corresponding studies in the murine system. However, immunofluorescence assays on human fetal thymic tissues showed that TCR δ and TCR β proteins are expressed at 9.5 weeks of gestation, at least in the cytoplasm, and TCR-αβ expression follows at 10 weeks. TCR δ protein expression peaks early at 9.5 weeks, falling to <1% of the total thymocyte population by 15 weeks of gestational age (*22*).

Anatomical Site of T Cell Receptor Rearrangements

Where does the rearrangement of the TCR α, β, γ, and δ genes occur, and where does the expression of the TCR-γδ and TCR-αβ receptors occur during T cell development? Expression of these genes and the receptor they encode must be considered in the context of T cell development. The initial differentiation of pluripotent stem cells in fetal yolk sac, bone marrow, and liver requires acquisition of terminal deoxynucleotidyl transferase (TdT, thought to be involved in N-segment nucleotide additions), cytoplasmic CD3, and some early T cell surface markers, such as CD7 (defined in man by the monoclonal antibody 3A1) or murine PGP-1 (equivalent to human A1G3 and Hermes-1) (*16, 23*). They, or other unidentified molecules, may function as homing receptors for those prothymocytes that colonize the fetal thymus at day 14 in the mouse (*24*) or at 8 to 9 weeks in man (*25*). In man, CD2 (T11, LFA-2, the sheep erythrocyte receptor) and in mouse, Thy-1 are the first known antigens acquired in the thymic environment. In man, $CD7^+$, cytoplasmic $CD3^+$ or $CD3^-$, T cell precursors colonize the thymic rudiment and then express CD2 (*22, 25*). Subsequent intrathymic maturation (Fig. 2) involves the acquisition and, in some cases, the loss of CD1 (T6), CD4, CD8, surface CD3, TCR-γδ or TCR-αβ, class I MHC antigens, and the lymphocyte homing receptor (Mel 14 in the mouse) (*16, 26*). Most of the exported immunocompetent T cells are positive for CD2, CD7, CD3, TCR-αβ, class I MHC, Mel 14, and either CD4 or CD8. A minority of T cells lack both CD4 and CD8 and express TCR-γδ. The earliest thymic immigrants are probably localized in the subcapsular region of the thymic cortex. They move through the cortex to the medulla and then are exported. During this process they encounter, in succession, thymic nurse cells in the outer region of the cortex, thymic dendritic cells in the inner region of the cortex, and medullary epithelial cells (which may further differentiate in the medulla to form Hassall's corpuscles), all of which are of epithelial origin and could themselves represent a continuous differentiation system (*27*). During this process, more than 99% of the T cell immigrants die, and the small remaining number, which are exported to the periphery, are self-tolerant; they do not recognize any of the normal components of self. This applies both to TCR-αβ–expressing T cells, which recognize foreign antigens only in association with self class I or class II MHC antigens, and to TCR-γδ cells, which can recognize some unidentified antigens under circumstances that have not been fully defined. There is some confusion, however, since in the mouse the lymphocyte homing receptor Mel 14, whose appearance may represent terminal differentiation of T lymphocyte development in the thymus, is found primarily on cortical rather than on medullary thymocytes (*26*). Thus, if the route described above is the maturational route for intrathymic T lymphocytes, then the mature medullary lymphocytes must reenter the cortex prior to export.

Many lines of evidence indicate that the thymus is the principal locus of T cell maturation (*28*). For example, thymic organ cultures have been used to study the maturation of stem cells (*29*). A single stem cell can recolonize the thymus and produce the full repertoire of T cells (*30*). Can maturation of TCR-γδ (and TCR-αβ) cells also occur at extrathymic sites? In man this problem is highlighted by current efforts at bone marrow transplantation for leukemias and lymphomas after all hematopoietic cells are deleted by irradiation and chemotherapy. In such chimeric individuals functional T cells are reconstituted, presumably in part because the thymic epithelium remains functional. However, in adults, because the thymus has involuted, reconstitution is more difficult and the frequency of graft versus host disease (lack of appropriate thymic education) is higher than in children. In the mouse, thymectomy, lethal irradiation, and subsequent transplantation with bone marrow depleted of $Thy\text{-}1^+$ cells results in chimeras whose $Thy\text{-}1^+$ precursors of cytotoxic T lymphocytes (CTLs) are able to generate a CTL repertoire without any apparent holes (*31*). Thus, maturation must occur at extrathymic sites.

Additional information is available from studies of the athymic nude mouse (*32*). This mutant animal has a rudimentary thymus and some defect in skin development manifested by the nude (hairless) phenotype. Both the thymus and the skin are epithelial in origin, and the skin may also have an important function in the immune system. If so, then the complex phenotype may be understandable as the result of a genetic defect affecting immune development in both of these epithelial organs.

Although the athymic nude mouse is grossly deficient in peripheral T cells, cells bearing T cell markers appear in increasing numbers as these animals age (*32*). Moreover, this phenomenon is more prominent when the genetic lesion is cross-bred into some mouse strains than into others. Some of these T cells clearly express TCR-αβ, although at reduced levels. However, these cells express an oligoclonal repertoire of TCR β genes (in which members of the $V_\beta 8$ family predominate) with limited functional potential. In one study, the amount of TCR γ-chain mRNA was several times higher than that found in a pooled population of T cells from normal mice. Four cDNA clones encoding TCR γ obtained from such mRNA all encoded full-length functional transcripts. The possibility that the rearrangement and expression of γ-chain genes might occur more efficiently in the athymic mouse was suggested. These animals also had increased numbers of immature $CD4^-CD8^-$ T cells, the phenotype of cells expressing TCR-γδ. Further studies are required to establish that these animals have cells expressing the TCR-γδ protein and to identify the site of the rearrangement and expression of the γ and δ genes. In this context one report (*14*) suggested the possibility that some γ-chain rearrangement might occur prethymically in the mouse fetal liver. However, this result was due to a partial restriction endonuclease cleavage evident as a single aberrant band in a Southern blot (*14*).

Thus, we are left with the view that the thymus is the principal locus of rearrangement of the TCR γ and presumably δ genes (as well as of α and β genes). However, some rearrangement probably occurs extrathymically at unknown sites, of which the bone marrow may be one (*33*). In normal animals the skin may be another organ in which such rearrangement can occur. But since the genetic defect in athymic mice also affects skin, it is possible that there is still another site, or alternatively that rearrangement may occur at a slow rate in many locations in the absence of factors derived from epithelial cells.

Lineages in T Cell Development in the Thymus

A complex developmental pathway in the thymus eventually leads to the formation of double-negative TCR-γδ–expressing cells and of $CD4^+CD8^-$ or $CD4^-CD8^+$ TCR-αβ–expressing cells, which are then exported to the periphery. An essential question is whether rearrangement of TCR γ and δ genes is obligatory for the rearrangement of TCR β and α genes and subsequent expression of a TCR-αβ receptor, or whether these two types of receptors represent totally different lineages.

Pluripotent stem cells, derived successively in the mouse from the yolk sac until about day 16 (day 60 in man), from fetal liver between days 12 and 20 (days 50 and 150 in man), or from the bone marrow after day 17 (day 79 in man) can colonize the thymus (pathway A in Fig. 2). These cells must enter the thymus with the use of a homing receptor in a process that also involves a small chemotactic peptide, thymotaxin, that is synthesized in the thymus (*34*). Early thymic markers that are candidates for the homing receptor are CD7 in man

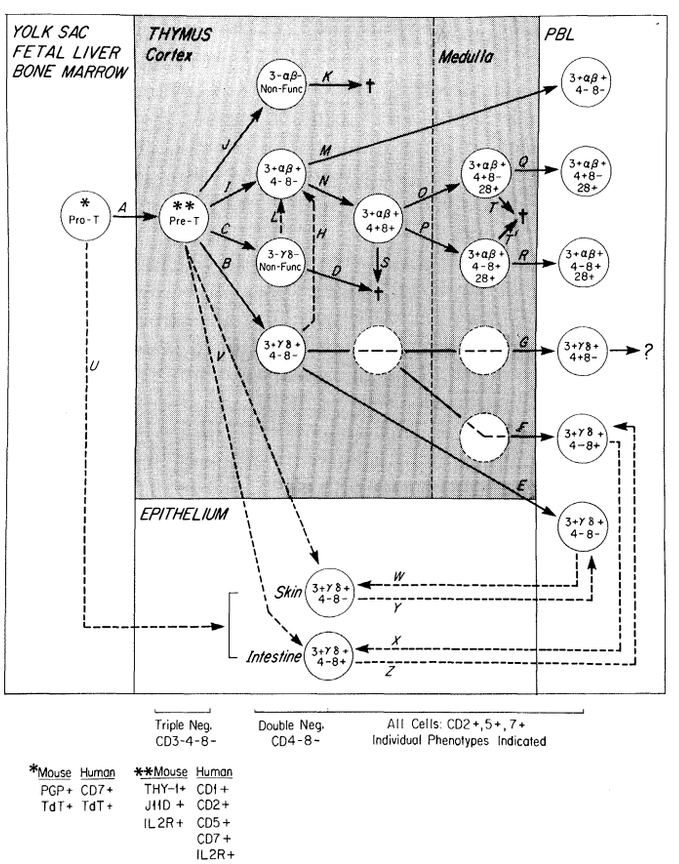

Fig. 2. Developmental pathways for TCR-bearing cells.

(no murine homolog has been identified) and PGP-1 (A1G3) (*23, 25*). Thymic development is actively driven by interactions with thymic epithelial cells and the factors they produce (*35*). Early in murine thymic development, Thy-1, J11D (B2A2), and the interleukin-2 receptor (IL-2R) appear in an unknown order. CD2 and CD1 are additional early thymocyte markers. Thus, in the mouse, $Thy\text{-}1^+$, $J11D^+$, $IL\text{-}2R^+$ thymocytes, and in man, $CD7^+$, $CD2^+$, $CD1^+$, and $IL\text{-}2R^+$ thymocytes, are early triple negative ($CD3^-CD4^-CD8^-$) precursors of more mature thymocytes (*16, 36*). The triple negative subset of thymocytes (less than 1% of the total) contains the precursors of all the mature subsets, as determined by reconstitution experiments in vivo and with organ cultures in vitro. These cells also express components of CD3 intracytoplasmically, although they do not express surface CD3. The exact time and place of expression of the components of the CD3 complex is not known, but at least some of these are thought to be expressed prethymically (*22, 37*). The proliferation of triple-negative thymocytes may be driven by interactions of CD2 with LFA-3 on thymic epithelial cells (*35*) as well as by IL-2 and IL-2R (*38*). Thymic epithelial cells secrete IL-1, granulocyte colony-stimulating factor, and myeloid colony-stimulating factor (*35*). A number of signals are undoubtedly required to move T cells through the proliferative cell cycle from G_0 to G_1, from G_1 through S to G_2, and finally through cell division, as in the case of proliferating B cells (*39*). Candidates for proliferation signals in triple-negative thymocytes include (i) CD2 and its ligand (LFA-3 or possibly soluble factors, although they have not been demonstrated) (*35, 40*), (ii) the IL-2R and IL-2, (iii) the IL-1R (not yet demonstrated on triple-negative thymocytes) and IL-1, (iv) Thy-1 (since antibodies against Thy-1 induce proliferation in the presence of IL-1) (*41*), and (v) IL-4 and granulocyte-macrophage colony-stimulating factor, whose receptors have not yet been identified (*42*). In addition, it seems likely that the proliferation signals for the maturational steps that lead to rearrangement of the TCR genes and expression of TCRs are distinct from those used later. In particular, stimulation through CD3 or TCR (or both), and possibly CD28, may become essential components of the proliferation process in more mature cells (*43, 44*).

The three types of TCR-expressing cells described above—that is, double-negative TCR-γδ cells, double-negative TCR-αβ cells, and the TCR-αβ cells expressing either CD4 or CD8 or both—develop from triple-negative ($CD3^-CD4^-CD8^-$) precursors. One possible scheme is shown in Fig. 2. At the earliest stage, some triple-negative thymocyte precursors must rearrange TCR γ and δ genes (and presumably TCR β genes, which rearrange at about the same time as TCR γ). Two general outcomes are possible: either both γ and δ genes are functionally rearranged, in which event a $CD3^+$ TCR-γδ–expressing cell is formed (pathway B), or either TCR γ or TCR δ or both are rearranged nonfunctionally, in which case no TCR or CD3 is expressed at this stage, possibly leading to cell death (pathways C and D). Somewhere in this lineage, CD5 begins to be expressed, and TCR-γδ–expressing cells, which are $CD3^+$, are also $CD5^+$. Similarly, at some point CD1 in human cells is expressed and then becomes silent again because $CD3^+$ TCR-γδ–expressing cells do not express CD1. Some or all of the cells that express surface CD3 and TCR-γδ exit the thymus and presumably appear in the periphery (pathway E). The manner and location in which a few such cells acquire CD8 and still fewer CD4 (pathways F and G) are unknown as is whether or not these cells undergo positive and negative thymic selection as do TCR-αβ–expressing cells. An intriguing question is whether any of the cells that express TCR-γδ can undergo further intrathymic maturation by rearrangement and expression of TCR α and β genes (pathway H), which would result simultaneously in deletion of a functionally rearranged TCR δ locus. This is possible, since some cells expressing TCR-αβ have residual functional TCR γ expression

(which, however, does not lead to surface expression of any known receptor), and by the dramatic decrease in the numbers of cells expressing TCR-γδ after TCR α rearrangement commences.

The predominant pathway for TCR-αβ expression appears to be rearrangement of the TCR α and β genes independent of rearrangement of TCR δ and possibly TCR γ genes (pathway I). When the TCR δ DNA that is deleted upon VJα joining in the murine thymus was isolated, all 400 excision circles obtained had Dδ2 and Jδ1 in the germline configuration (*45*). However, all murine (and human) TCR-αβ–expressing T cell clones examined so far [with one exception (*46*)] have rearranged the γ locus; the majority express a nonfunctional γ transcript, but a few express a functional γ mRNA. Studies of TCR β transgenic animals also suggest the independence of the two TCR pathways (*47*). If the TCR α or β genes are nonfunctionally rearranged at this stage, presumably the cells are unable to proliferate and die (pathways J and K).

An alternative pathway for TCR-γδ cells could occur, although no direct evidence for it exists, and earlier it was thought to be the predominant pathway; the cells that have nonfunctional rearrangements of TCR γ and δ might undergo further rearrangement of the TCR α and β genes, also leading to the $CD4^-CD8^-$ TCR-αβ–expressing cells (pathway L). However, functional rearrangement of both α and β genes by pathways I or L leads to expression of the unusual $CD4^-CD8^-$ (double-negative) $CD3^+$ TCR-αβ–expressing cells, whose rearrangements at the TCR β locus are restricted to the $V_\beta 8$ gene segment. Small numbers of these cells may be in the periphery, but in *lpr* or *gld* mice they accumulate in the periphery in large numbers (pathway M).

In normal mice the mainstream of maturation of T cells (pathways N, O, and P, for which there are alternatives) leads to $CD3^+CD4^+CD8^-$ and $CD3^+CD4^-CD8^+$ TCR-αβ–expressing cells in the periphery (pathways Q and R). Much recent data suggest that $CD3^+CD4^+CD8^+$ TCR-αβ–expressing cells (which represent most thymocytes) are intermediates in this differentiation pathway, and are the substrate for positive selection; cells that are not selected die (pathway S). All $CD3^+$ TCR-αβ–expressing cells, whether double-negative or CD4- or CD8-positive, express CD5. The maturation of the TCR-αβ–expressing cells also requires the acquisition of CD28, which may be another activation receptor for TCR-αβ cells (*44*). In addition, many cells that express functional TCR-αβ rearrangements and are $CD3^+$ TCR-αβ–expressing cells are eliminated as a result of negative thymic selection (pathways T and T^1).

Finally, some comment should be made on the origin of the $CD3^+$ TCR-γδ–expressing cells found in skin and intestinal epithelia. Three origins can be envisaged (Fig. 2): (i) a prothymocyte migrates to the epithelial tissues and matures there (pathway U); (ii) partial maturation occurs in the thymus, and a pre-T cell migrates to and completes its maturation in the epithelial tissues (pathway V); and (iii) $CD3^+$ TCR-γδ–expressing cells mature in the thymus, exit, and reach the epithelial tissues through the blood (pathways W and X). Reciprocally, if maturation of $CD3^+$ TCR-γδ–expressing cells can occur in the skin and intestinal epithelia, then some of the $CD3^+$ TCR-γδ cells in blood may originate in these epithelia (pathways Y and Z). Thus, it is clear that TCR-bearing cells have complex lineages, and much remains to be learned.

Most of these studies were done in the murine system, although recently some important advances have been made in human studies. In addition to studies of fetal material, studies that have been done so far in postnatal man, including investigations of neonatal thymocytes and of stage-specific leukemias and the T cell lines derived from them, do not contradict this scheme (*25, 48*). An immunodeficient mouse strain can be colonized with either human hematopoietic cell precursors or mature T cells (*49*), which suggests that human thymocyte maturation can now be studied in an animal model; rapid progress is therefore anticipated.

Mechanisms of Rearrangement and Expression of TCR Genes

Little is known about the details of rearrangement and expression of these genes. The processes involved are comparable to those of immunoglobulin (Ig) gene rearrangement and expression. A number of individual steps must occur: (i) the rearrangement of VJ segments in the γ locus, of VDJ segments in the δ locus, and of DJ segments in the β locus, followed later by VDJ β rearrangements; (ii) the transcription (and translation) of the rearranged γ and δ genes, which must depend on the expression of specific nuclear factors regulating the T cell–specific transcription of these genes; and (iii) a process analogous to switch recombination, which results both in rearrangement of the Vα and Jα gene segments to a configuration suitable for transcription and in deletion of the δ locus. This latter rearrangement may be preceded by a DNA deletional event, by transcriptional activity initiated in the Jα region, or both (*50*). These processes are then analogous to (i) V(D)J gene rearrangement at the Ig loci; (ii) the production of specific nuclear factors required for Ig gene transcription; and (iii) switch recombination, leading for example, to cessation of IgM synthesis and initiation of IgA synthesis. Little is known about the mechanisms or factors involved in the V(D)J recombination system, although because gene constructions that contain T cell receptor gene segments undergo recombination when introduced into pre-B cells, the recombinases that function in V(D)J rearrangement in B cells may be similar or identical to those in T cells (*51*). The nuclear factors that regulate the specific transcription of genes at the Ig heavy chain and the κ and λ loci have been identified at least in part and are under intense investigation. Similar factors that bind to the enhancers of TCR genes are being studied (*52*). Switch recombination that involves secretion of IgA has received renewed impetus with the discovery that the lymphokine IL-6 is a specific regulator of this recombinational process. It seems possible that some lymphokines also regulate TCR gene rearrangements.

Potentially important clues to mechanisms involved in TCR development may derive from studies of *lpr* (lymphoproliferation) and phenotypically similar *gld* (generalized lymphoproliferative disease) mice as well as other immunodeficient mouse strains (*53*). In homozygous *lpr* or *gld* animals, there is massive accumulation of lymphocytes in peripheral lymphoid organs. These cells are of T cell origin and are $Thy1^+CD5^+$, but $CD4^-CD8^-$ (double-negatives). They express low levels of CD3 and TCR-αβ receptors, and they anomalously express two B cell markers, B220 and PC.1, although they do not rearrange Ig genes (*54*). The lymphoaccumulation is abrogated by thymectomy and restored by implantation of either an *lpr* thymus or a normal thymus. Thus, the genetic defect must lie in the developing T cells rather than in the thymus. The two genetic defects, *lpr* and *gld*, are not allelic, however, The effects of the mutations differ in several respects, including the time of survival, which may be related to the nature of the autoimmune process. Mice with the *lpr* mutation develop a lupus-like syndrome involving necrotizing arteritis, glomerular nephritis, and perivascular lymphoid infiltration, whereas *gld* mice are essentially free from this histopathology but develop instead interstitial pneumonitis together with antinuclear antibodies and thymocyte-binding autoantibodies. Both types of mutation, however, result in a lymphoproliferation (more properly termed lymphoaccumulation, since the vast majority of these lymph node cells are not dividing). The phenotype of these cells resembles that of the double-negative TCR-αβ–expressing cells

recently detected in normal thymus (pathway I or L in Fig. 2), except that the latter do not express B cell markers. A plausible explanation of the lymphoaccumulation would take account of the fact that 99% of T cells that enter the normal thymus die. In this model, the entry of cells into pathway S is blocked in both mutations; this could also result from a reduction in pathways B, C, and N which may be rate-limiting. T cells, which would thus be shunted into pathway M, would exit the thymus in very large numbers and accumulate in peripheral lymphoid organs. The large accumulation of peripheral cells must result from failure of intrathymic cell death.

The only clue to a possible genetic defect in *lpr* cells is that even though the accumulating cells have few TCR γ-chain transcripts (although the TCR γ-chain gene is rearranged), transcripts increase rapidly more than 200 times when the cells are treated with phorbol esters (*55*). No such effect is seen on similar treatment of the double-negative TCR-αβ–expressing cells obtained from normal thymocytes. One explanation for this observation is that the abnormal cells lack some element required for expression of a nuclear factor that regulates TCR γ-chain expression from the rearranged TCR γ-chain gene (for example, a defect in a receptor for a lymphokine or a defect in the production of that lymphokine itself). Induction by phorbol ester would bypass the defect. The hypothesis that the *lpr* and *gld* "mutations may affect different enzymes in a common metabolic pathway of major importance to T cell differentiation and function" (*56*) could be restated as "*lpr* and *gld* mutations may affect different transacting nuclear factors required for TCR γ gene expression." In any event, the further study of these mutations will provide important information about T cell development.

Kinetics of Expression of T Cell Receptors: Some Unanswered Questions

What is the explanation for the high level of TCR-γδ–expressing cells after day 14 in fetal development? This question should be coupled with a second query. Why is α-chain rearrangement delayed several days after γδ (and β) chain rearrangement in the mouse? Both positive and negative selection theories can be considered to explain the high level of TCR-γδ–expressing cells at days 15 and 16. One possibility for positive selection is that at this time in fetal development some factor exists that drives proliferation of TCR-γδ cells. A second (unlikely) possibility is that some special mechanisms ensure that the N nucleotides added always result in functional transcription; no precedent for such a mechanism exists. A more likely possibility is simply that the percentage of TCR-γδ cells is abnormally high because the expression of TCR-αβ has not yet begun. The subsequent expansion of TCR-αβ–expressing cells would dilute the high number of TCR-γδ–expressing cells that appeared earlier. Moreover, some of these TCR-γδ–expressing cells could be eliminated, even though the transcripts were functional, by rearrangement of the TCR α locus in these cells and consequent deletion of TCR δ; in fact, the first TCR γ cDNA described in a murine TCR-αβ–expressing cell encoded a functional transcript (*5*). In any event, a small percentage of TCR-γδ cells reach the periphery even after TCR α rearrangement begins.

What signals TCR α rearrangement and why is it delayed? The rearrangement of TCR α, accompanied by deletion of TCR δ, occurs at day 16 in the mouse, several days after β, γ, and δ rearrangements and after expression of TCR-γδ. Nothing is known about the mechanism of α rearrangement, whether it involves appearance of a lymphokine at that time of development or interaction with a particular cell surface molecule in the thymus. This mechanism is a central question in developmental immunology. Although α rearrangement is analogous to isotype switching in B cells, mechanistically it involves VJα joining and thus is quite similar to the VDJδ joining that occurs earlier in the same genetic region. By what means are these two processes distinguished? The heptamer and nonamer joining signals that surround these segments are indistinguishable in α and δ gene segments (*57*) and thus the simple hypothesis that sequence differences in these signals are involved in the mechanisms can be discarded.

Why is TCR-αβ expression delayed by several days in comparison to TCR-γδ expression? Again, no answer is available, but the possibility that this delay ensures flooding of the periphery with cells expressing TCR-γδ at a particular time in development requires careful evaluation. The circulation of these cells in the fetus could have some special role in fetal immunology. A second possibility, however, might be that high level TCR-γδ expression is required to seed epithelial tissues at a particular time in development. The epithelium of the organism, whether the skin, the gut, or the pulmonary epithelium, is the primary barrier to invasion by foreign organisms. Several special types of cells have been found in the skin epithelium of both man and mouse. Langerhans cells are MHC class II^+ $CD1^+$ in human cells and Thy-1^- in mouse cells. They may be the skin homolog of macrophages, whose primary function is to present foreign antigens. It is unclear what role CD1 (a non-HLA class I protein) might have in this function. In addition, dendritic epithelial cells occur in the mouse and are Thy-$1^+$$CD3^+$, $WT31^-$ cells expressing TCR-γδ (*58, 59*). In man, however, although TCR-γδ lymphocytes have recently been observed in skin, they have a normal morphology and they do not appear to accumulate specifically in skin (*60*). The origin of these TCR-γδ cells is not known. They might arise from cells that mature in the thymus, exit the thymus, and subsequently seed the epithelium. Epithelia other than thymic epithelia, however, may also serve a differentiating function (*61*). Thus, some stem cells may migrate to the epithelial organs and differentiate there into TCR-γδ cells. TCR-γδ cells are also in gut epithelium (*62*) and might occur in other epithelia. Understanding the role of these cells in the epithelium will broaden our understanding of the immune system.

Thus, the discovery of cells bearing TCR-γδ that arise early in ontogeny has helped to lay a framework within which thymocyte development is being studied. The task of understanding mechanistically the various differentiation steps shown in Figs. 1 and 2, a central task of developmental immunology, will occupy molecular immunologists for a long time. However, in the process clues will be obtained that will allow biochemical study of the positive and negative thymocyte selection that leads to the death of more than 99% of the cells entering the thymus. At the same time this selection ensures the development of a peripheral immune system with an enormous repertoire and in which autoimmune attack on autologous tissues is relatively rare. As a consequence of understanding the mechanisms of this exquisite regulation, a more profound understanding of autoimmune disease is certain to emerge (*63*).

REFERENCES AND NOTES

1. H. L. Ploegh, H. T. Orr, J. L. Strominger, *Cell* **24**, 287 (1981); J. F. Kaufman, C. Auffray, A. J. Korman, D. A. Shackelford, J. L. Strominger, *ibid.* **36**, 1 (1984); P. J. Bjorkman *et al.*, *Nature* **329**, 506 (1987); *ibid.*, p. 512.
2. A. S. Rosenthal and E. M. Shevach, *J. Exp. Med.* **138**, 1194 (1973); D. H. Katz, T. Hamaoka, M. E. Dorf, B. Benacerraf, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2631 (1973); R. N. Zinkernagel and P. C. Doherty, *Nature* **251**, 547 (1974).
3. Z. Dembic *et al.*, *Nature* **320**, 232 (1986); T. Saito *et al.*, *ibid.* **325**, 125 (1987).
4. M. Kronenberg, G. Siu, L. E. Hood, N. Shastri, *Annu. Rev. Immunol.* **4**, 529 (1986); J. P. Allison and L. L. Lanier, *ibid.* **5**, 503 (1987); B. Toyonaga and T. W. Mak, *ibid.* **5**, 585 (1987); H. von Boehmer, *ibid.* **6**, 309 (1988); O. Acuto and L. M. Larocca, *Human Immunol.* **18**, 93 (1987).
5. H. Saito *et al.*, *Nature* **309**, 757 (1984); D. H. Raulet, R. D. Garman, H. Saito, S. Tonegawa, *ibid.* **314**, 103 (1985).

6. C. Murre *et al.*, *ibid.* **316**, 549 (1985); T. H. Rabbitts *et al.*, *EMBO J.* **4**, 1461 (1985); D. P. Dialynas *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2619 (1986).
7. M. B. Brenner, J. L. Strominger, M. S. Krangel, *Adv. Immunol.* **43**, 133 (1988); M. B. Brenner *et al.*, *Nature* **322**, 145 (1986); Y. Chien, M. Iwashima, K. B. Kaplan, J. F. Elliott, M. M. Davis, *ibid.* **327**, 677 (1987); S. Hata, M. B. Brenner, M. S. Krangel, *Science* **238**, 678 (1987); E. Y. Loh *et al.*, *Nature* **330**, 569 (1987); D. Raulet, *Annu. Rev. Immunol.* **7**, 175 (1989).
8. C. L. Chen, J. Cihak, U. Losch, M. D. Cooper, *Eur. J. Immunol.* **18**, 539 (1988); J. T. Sowder, C. H. Chen, L. L. Ager, M. M. Chan, M. D. Cooper, *J. Exp. Med.* **167**, 315 (1988).
9. H. R. Snodgrass, Z. Dembic, M. Steinmetz, H. von Boehmer, *Nature* **315**, 232 (1985); H. R. Snodgrass, P. Kisielow, M. Kiefer, M. Steinmetz, H. von Boehmer, *ibid.* **313**, 592 (1985).
10. J. A. Bluestone, D. Pardoll, S. O. Sharrow, B. J. Fowlkes, *ibid.* **326**, 82 (1987); D. M. Pardoll *et al.*, *ibid.*, p. 79.
11. Y. Yoshikai, Y. Yanagi, N. Suciu-Foca, T. W. Mak, *ibid.* **310**, 506 (1984); T. Lindsten, B. J. Fowlkes, L. E. Samelson, M. M. Davis, Y. Chien, *J. Exp. Med.* **166**, 761 (1987); Y. Chien, M. Iwashima, K. B. Kaplan, J. F. Elliott, M. M. Davis, *Nature* **327**, 677 (1987).
12. W. Born, G. Rathbun, P. Tucker, P. Marrack, J. Kappler, *Science* **234**, 479 (1986).
13. N. Roehm *et al.*, *Cell* **38**, 577 (1984); W. Born, J. Yague, E. Palmer, J. Kappler, P. Marrack, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2925 (1985); M. K. L. Collins *et al.*, *ibid.*, p. 4503 (1985); A. G. Farr, S. K. Anderson, P. Marrack, J. Kappler, *Cell* **43**, 543 (1985); J. S. Heilig *et al.*, *Nature* **317**, 68 (1985); D. M. Kranz *et al.*, *ibid.* **313**, 752 (1985); L. E. Samelson *et al.*, *ibid.* **315**, 765 (1985); A. Cristanti, A. Colantoni, R. Snodgrass, H. von Boehmer, *EMBO J.* **5**, 2837 (1986); R. D. Garman, P. J. Doherty, D. H. Raulet, *Cell* **45**, 733 (1986); J. S. Heilig and S. Tonegawa, *Nature* **322**, 836 (19869; B. Jones, S. Mjolsness, C. Janeway, Jr., A. C. Hayday, *ibid.* **323**, 635 (1986); E. B. Reilly, D. M. Kranze, S. Tonegawa, H. N. Eisen, *ibid.* **321**, 878 (1986); F. Rupp, G. Frech, H. Hengartner, R. M. Zinkernagel, R. Joho, *ibid.*, p. 876; A. M. Lew *et al.*, *Science* **234**, 1401 (1987); T. Quertermous, W. M. Strauss, J. J. M. van Dongen, J. G. Seidman, *J. Immunol.* **138**, 2687 (1987); Y. Yoshikai *et al.*, *Eur. J. Immunol.* **17**, 119 (1987); F. Triebel, M. P. Lefranc, T. Hercend, *ibid.* **18**, 789 (1988).
14. R. Haars *et al.*, *J. Exp. Med.* **164**, 1 (1986); R. Haars and L. Hood, personal communication.
15. D. M. Asarnow *et al.*, *Cell* **55**, 837 (1988); G. Steiner *et al.*, *Eur. J. Immunol.* **18**, 1323 (1988).
16. B. J. Mathieson and B. J. Fowlkes, *Immunol. Rev.* **82**, 141 (1984); R. Scollay, P. Bartlett, K. Shortman, *ibid.*, p. 79; B. Adkins *et al.*, *Annu. Rev. Immunol.* **5**, 325 (1987); L. Smith, *Nature* **326**, 798 (1987); H. S. Teh *et al.*, *ibid.* **325**, 229 (1988); W. C. Sha *et al.*, *ibid.*, p. 271.
17. J. W. Kappler, N. Roehm, P. Marrack, *Cell* **49**, 273 (1987); J. W. Kappler, U. D. Staerz, J. White, P. Marrack, *Nature* **332**, 35 (1988); P. Kisielow, H. Bluthmann, U. D. Staerz, M. Steinmetz, H. von Boehmer, *ibid.*, **333**, 742 (1988); H. R. MacDonald *et al.*, *ibid.* **332**, 40 (1988); W. C. Sha *et al.*, *ibid.* **335**, 271 (1988); P. Kisielow, H. S. Teh, H. Bluthmann, H. von Boehmer, *ibid.*, p. 730; A. M. Pullen, P. Marrack, J. W. Kappler, *ibid.*, p. 796; J. White *et al.*, *Cell* **56**, 27 (1989).
18. R. C. Budd *et al.*, *J. Exp. Med.* **166**, 577 (1987); I. N. Crispe *et al.*, *Nature* **329**, 336 (1987); B. J. Fowlkes *et al.*, *ibid.*, p. 251.
19. V. Groh *et al.*, *Fed. Proc.* **3**, A485 (1989); *Proc. Natl. Acad. Sci. U.S.A.*, in press.
20. C. H. Chen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press (1989).
21. F. V. Jottereau and N. M. LeDouarin, *J. Immunol.* **129**, 1869 (1982); N. M. Le Douarin, *Curr. Top. Dev. Biol.* **20**, 291 (1986); M. Cooper and N. M. LeDouarin, personal communication.
22. B. F. Haynes, K. H. Singer, S. M. Denning, M. E. Martin, *J. Immunol.* **141**, 3776 (1988); D. Campana *et al.*, *ibid.* **142**, 57 (1989).
23. B. F. Haynes *et al.*, *ibid.* **131**, 1195 (1983); M. S. Hershfield *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 253 (1984); J. Kurtzberg, S. H. Bigner, M. S. Hershfield, *J. Exp. Med.* **162**, 1561 (1985); I. S. Trowbridge, J. Lesley, J. Trotter, R. Hyman, *Nature* **315**, 666 (1985); E. S. Medlock, I. Goldschneider, D. L. Greiner, L. Shultz, *J. Immunol.* **138**, 3590 (1987); R. Palacios *et al.*, *J. Exp. Med.* **166**, 12 (1987); B. F. Haynes, M. E. Martin, H. Kay, J. Kurtzberg, *ibid.* **168**, 1061 (1988); J. Kurtzberg *et al.*, *Blood* **73**, 381 (1989); L. Picker, J. de los Toyos, M. Telen, B. F. Haynes, E. Butcher, *J. Immunol.* **142**, 2046 (1989).
24. M. A. S. Moore and J. J. T. Owen, *J. Exp. Med.* **126**, 715 (1967); J. C. Fontaine-Perus, F. M. Calman, C. Kaplan, N. M. LeDouarin, *J. Immunol.* **126**, 2310 (1981).
25. D. F. Lobach, L. L. Hensley, W. Ho, B. F. Haynes, *J. Immunol.* **135**, 1752 (1985); B. F. Haynes, *Clin. Res.* **34**, 422 (1986); D. F. Lobach and B. F. Haynes, *J. Clin. Immunol.* **7**, 81 (1987); B. F. Haynes, S. M. Denning, K. H. Singer, J. Kurtzberg, *Immunol. Today* **10**, 87 (1989); S. Jalkanen, N. Wu, R. F. Bargatze, E. C. Butcher, *Annu. Rev. Med.* **38**, 467 (1987).
26. P. J. Fink, W. M. Gallatin, R. A. Reichert, E. C. Butcher, I. L. Weissman, *Nature* **313**, 233 (1985).
27. D. F. Lobach, R. M. Scearce, B. F. Haynes, *J. Immunol.* **134**, 250 (1985); A. J. Laster, T. Itoh, T. J. Palker, B. F. Haynes, *Differentiation* **31**, 67 (1986); D. F. Lobach, T. Itoh, K. H. Singer, B. F. Haynes, *ibid.* **34**, 50 (1987).
28. O. Stutman, *Clin. Immunol. Allergy* **5**, 191 (1985).
29. P. Kisielow, W. Leiserson, H. von Boehmer, *J. Immunol.* **133**, 1117 (1984); M. J. Owen, E. J. Jenkinson, G. T. Williams, R. Kingston, J. J. T. Owen, *Eur. J. Immunol.* **16**, 875 (1986); W. Born *et al.*, *J. Immunol.* **138**, 999 (1987); R. Eren, D. Zharhary, L. Abel, A. Globerson, *Cell. Immunol.* **108**, 76 (1987).
30. R. Kingston, E. J. Jenkinson, J. J. T. Owen, *Nature* **317**, 811 (1985); G. T. Williams, R. Kingston, M. J. Owen, E. J. Jenkinson, J. J. T. Owen, *ibid.* **324**, 63 (1986); S. Ezine, L. Jerabek, I. Weissman, *J. Immunol.* **139**, 2195 (1987).
31. V. Duprez, B. Hamilton, S. J. Burakoff, *J. Exp. Med.* **156**, 844 (1982).
32. H. R. MacDonald, C. Blanc, R. K. Lees, B. Sordat, *J. Immunol.* **136**, 4337 (1986); Y. Yoshikai, M. D. Reis, T. W. Mak, *Nature* **324**, 482 (1986); H. R. MacDonald, R. K. Lees, C. Bron, B. Sordat, G. Miescher, *J. Exp. Med.* **166**, 195 (1987); J. R. Maleckar and L. A. Sherman, *J. Immunol.* **138**, 3873 (1987); D. M. Pardoll *et al.*, *ibid.* **140**, 4091 (1988).
33. R. Chervenak, J. W. Moorhead, J. J. Cohen, *J. Immunol.* **134**, 695 (1985); M. Ahmad, J. C. Lecron, J. J. Descombe, J. Tanzer, P. Goube de Laforest, *Cell. Immunol.* **103**, 299 (1986); J. L. Hurwitz, J. Samaridis, J. Pelkonen, *Cell* **52**, 821 (1988); P. Benveniste, B. S. Chadwick, R. G. Miller, *J. Exp. Med.*, in press.
34. B. A. Imhof *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7699 (1988).
35. K. H. Singer *et al.*, *ibid.* **83**, 6588 (1986); S. M. Denning, D. T. Tuck, K. H. Singer, B. F. Haynes, *J. Immunol.* **138**, 680 (1987); S. M. Denning *et al.*, *ibid.* **139**, 2573 (1987); P. T. Le, D. Tuck, C. A. Dinarello, B. F. Haynes, K. H. Singer, *ibid.* **138**, 2520 (1987); S. Mizutani *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4999 (1987); M. Ogata *et al.*, *J. Immunol.* **139**, 2675 (1987); S. M. Denning *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 3125 (1988); P. T. Le *et al.*, *J. Immunol.* **141**, 1211 (1988).
36. D. F. Lobach, L. L. Hensley, W. Ho, B. F. Haynes, *J. Immunol.* **135**, 1752 (1985); E. Rothenberg and J. P. Lugo, *Dev. Biol.* **112**, 1 (1985); A. De La Hera *et al.*, *Eur. J. Immunol.* **16**, 653 (1986); M. L. Toribio *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6985 (1986); I. N. Crispe and M. J. Bevan, *J. Immunol.* **138**, 2013 (1987).
37. D. Campana, J. S. Thompson, P. Amlot, S. Brown, S., G. Janossy, *J. Immunol.* **138**, 648 (1987); A. Tunnacliffe, L. Buluwela, T. H. Rabbitts, *EMBO J.* **6**, 2953 (1987); J. J. M. van Dongen *et al.*, *J. Immunol.* **138**, 1260 (1987).
38. R. Ceredig, J. W. Lowenthal, M. Nabholz, H. R. MacDonald, *Nature* **314**, 98 (1985); A. De La Hera, M. L. Toribio, C. Marquez, C. Martinez-A., *Proc. Natl. Acad. Sci. U.S.A.* **82**, 6268 (1985); S. Habu, K. Okumura, T. Diamantstein, E. M. Shevach, *Eur. J. Immunol.* **15**, 456 (1985); J. P. Lugo *et al.*, *J. Exp. Med.* **161**, 1048 (1985); D. H. Raulet, *Nature* **314**, 100 (1985); H. von Boehmer, A. Crisanti, P. Kisielow, W. Haas, *ibid.*, p. 539; J. P. Lugo, S. N. Krishnan, R. D. Sailor, E. V. Rothenberg, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1862 (1986); R. Palacios and H. von Boehmer, *Eur. J. Immunol.* **16**, 12 (1986); A. De La Hera, M. L. Toribio, M. A. R. Marcos, C. Marquez, C. Martinez-A., *ibid.* **17**, 683 (1987); E. J. Jenkinson, R. Kingston, J. J. T. Owen, *Nature* **329**, 160 (1987); K. L. McGuire and E. V. Rothenberg, *EMBO J.* **6**, 939 (1987); R. P. Shimonkevitz, L. A. Husmann, M. J. Bevan, I. N. Crispe, *Nature* **329**, 157 (1987).
39. W. Lernhardt *et al.*, *Immunol. Rev.* **99**, 241 (1987).
40. D. A. Fox *et al.*, *J. Immunol.* **134**, 330 (1985); E. L. Reinherz, *Immunol. Today* **6**, 75 (1985); M. L. Blue, J. F. Daley, H. Levine, K. A. Craig, S. F. Schlossman, *J. Immunol.* **138**, 3108 (1987); G. H. Reem, S. Carding, E. L. Reinherz, *ibid.*, **139**, 130 (1987).
41. R. Palacios, P. Sideras, H. von Boehmer, *EMBO J.* **1**, 91 (1987); P. Sideras and R. Palacios, *Eur. J. Immunol.* **17**, 217 (1987); H. Spits *et al.*, *J. Immunol.* **139**, 1142 (1987); L. Tentori *et al.*, *ibid.* **140**, 1089 (1988); H. R. MacDonald, G. C. Miescher, R. C. Howe, *Eur. J. Immunol.* **17**, 1677 (1987).
42. K. H. Grabstein *et al.*, *J. Immunol.* **139**, 1148 (1987); T. Kupper, P. Flood, D. Coleman, M. Horowitz, *ibid.* **138**, 4288 (1987); A. Zlotnik, J. Ransom, G. Frank, M. Fischer, M. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3856 (1987).
43. C. D. Tsoukas *et al.*, *J. Immunol.* **90**, 3885 (1987); S. Marusic-Galesic *et al.*, *ibid.* **140**, 411 (1987); S. Y. Yang, S. M. Denning, S. Mizuno, B. Dupont, B. F. Haynes, *J. Exp. Med.* **168**, 1457 (1988).
44. A. Moretta *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1654 (1987).
45. A. Winoto and D. Baltimore, *Nature* **338**, 430 (1989).
46. M. Hattori, T. Sudo, H. Izawa, S. Kano, N. Minato, *Int. Immunol.*, in press.
47. H. von Boehmer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9729 (1988); L. J. Berg *et al.*, *Mol. Cell. Biol.* **8**, 5459 (1988).
48. E. L. Reinherz, P. C. Kung, G. Goldstein, R. H. Levey, S. F. Schlossman, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1588 (1980); G. Janossy, N. Tidman, E. S. Papageorgiou, P. C. Kung, G. Goldstein, *J. Immunol.* **126**, 1608 (1981); O. Acuto *et al.*, *Cell* **34**, 717 (1983); H. D. Royer *et al.*, *ibid.* **39**, 261 (1984); D. L. Williams *et al.*, *ibid.* **36**, 101 (1984); M. P. Link, S. J. Stewart, R. A. Warnke, R. Levy, *J. Clin. Invest.* **76**, 248 (1985); H. D. Royer, D. Ramarli, O. Acuto, T. J. Campen, E. L. Reinherz, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5510 (1985); M. P. Davey *et al.*, *ibid.* **83**, 8759 (1986); A. J. Furley *et al.*, *Cell* **46**, 75 (1986); R. N. Sangster, J. Minowada, N. Suciu-Foca, M. Minden, T. W. Mak, *J. Exp. Med.* **163**, 1491 (1986); Z. Chen *et al.*, *ibid.* **165**, 1000 (1987); C. A. Felix *et al.*, *J. Clin. Invest.* **80**, 545 (1987); B. Freimark, L. Lanier, J. Phillips, T. Quertermous, R. Fox, *J. Immunol.* **138**, 1724 (1987); R. Gonzalez-Sarmiento *et al.*, *J. Clin. Invest.* **79**, 1281 (1987); R. Goorha *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4547 (1987); L. J. Picker, M. B. Brenner, L. M. Weiss, S. D. Smith, R. A. Warnke, *Am. J. Pathol.* **129**, 434 (1987); S. Pittaluga, M. Uppenkamp, J. Cossman, *Blood* **69**, 1062 (1987).
49. J. M. McCune *et al.*, *Science* **241**, 1632 (1988); D. E. Mosier, R. J. Gulizia, S. M. Baird, D. B. Wilson, *Nature* **335**, 256 (1988).
50. J.-P. de Villartay *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8608 (1987); R. D. Hockett *et al.*, *ibid.* **85**, 9694 (1988); J.-P. de Villartay, R. D. Hockett, D. Coran, S. J. Korsmeyer, D. I. Cohen, *Nature* **335**, 170 (1988).
51. G. B. Yancopoulos, T. K. Blackwell, H. Suh, L. Hood, F. W. Alt, *Cell* **44**, 251 (1986).
52. S. Luria, G. Gross, M. Horowitz, D. Givol, *EMBO J.* **6**, 3307 (1987); H. D. Royer and E. L. Reinherz, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 232 (1987); P. Krimpenfort *et al.*, *EMBO J.* **7**, 745 (1988); S. McDougall, C. L. Peterson, K. Calame, *Science* **241**, 205 (1988); A. Winoto and D. Baltimore, *EMBO J.* **8**, 729 (1989).
53. J. B. Roths, E. D. Murphy, E. M. Eicher, *J. Exp. Med.* **159**, 1 (1984); D. Wofsy, R. R. Hardy, W. E. Seaman, *J. Immunol.* **132**, 2686 (1984); W. F. Davidson, K. L. Holmes, J. B. Roths, H. C. Morese, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 1219 (1985); D. A. Nemazee, S. Studer, M. Steinmetz, Z. Dembic, M. Kiefer, *Eur. J.*

Immunol. **15**, 760 (1985); Y. Hashimoto, A. M. Maxam, M. I. Greene, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7865 (1986); L. E. Samelson, W. F. Davidson, H. C. Morse III, R. D. Klausner, *Nature* **324**, 674 (1986); L. D. Shultz and C. L. Sidman, *Annu. Rev. Immunol.* **5**, 367 (1987); S. Yokota *et al.*, *J. Immunol.* **139**, 2810 (1987).
54. R. C. Budd, M. Schreyer, G. C. Miescher, H. R. MacDonald, *J. Immunol.* **139**, 2200 (1987).
55. G. C. Miescher *et al.*, *ibid.* **138**, 1959 (1987).
56. W. F. Davidson, F. J. Dumont, H. G. Bedigian, B. J. Fowlkes, H. C. Morse, *ibid.* **136**, 4075 (1986).
57. K. Satyanarayana *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8166 (1988).
58. F. Koning *et al.*, *Science* **236**, 834 (1987); W. A. Kuziel *et al.*, *Nature* **328**, 263 (1987).
59. G. Stingl *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2430 (1987); G. Stingl *et al.*, *ibid.*, p. 4586.
60. V. Groh *et al.*, *J. Exp. Med* **169**, 1277 (1989).
61. M. R. Rubenfeld *et al.*, *J. Invest. Dermatol.* 77, 221 (1981); T. S. Kupper, D. L. Coleman, J. McGuire, D. Goldminz, M. C. Horowitz, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4451 (1986).
62. R. P. Bucy, C-L. H. Chen, J. Cihak, U. Losch, M. D. Cooper, *J. Immunol.* **141**, 2200 (1988); T. Goodman and L. Lefrancois, *Nature* **333**, 855 (1988); C. Janeway, *ibid.*, p. 804; M. Bonneville *et al.*, *ibid.* **336**, 479 (1989).
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Research Articles

Three-Dimensional Spherical Models of Convection in the Earth's Mantle

DAVE BERCOVICI, GERALD SCHUBERT, GARY A. GLATZMAIER

Three-dimensional, spherical models of mantle convection in the earth reveal that upwelling cylindrical plumes and downwelling planar sheets are the primary features of mantle circulation. Thus, subduction zones and descending sheetlike slabs in the mantle are fundamental characteristics of thermal convection in a spherical shell and are not merely the consequences of the rigidity of the slabs, which are cooler than the surrounding mantle. Cylindrical mantle plumes that cause hotspots such as Hawaii are probably the only form of active upwelling and are therefore not just secondary convective currents separate from the large-scale mantle circulation. Active sheetlike upwellings that could be associated with mid-ocean ridges did not develop in the model simulations, a result that is in agreement with evidence suggesting that ridges are passive phenomena resulting from the tearing of surface plates by the pull of descending slabs.

OVER TIME SCALES OF MILLIONS OF YEARS AND LONGER, the earth's mantle behaves like a fluid. As the earth cools, the mantle fluid removes heat from the deep interior by the transport of mass, that is, by thermal convection. Mantle convection is generally accepted as the engine of plate tectonics (*1*) in that the rigid plates on the surface of the earth, whose relative motions create mountains, volcanoes, and earthquakes, are believed to be an integral part of the convective system and the surface expression of mantle convection. Accordingly, major plate tectonic features such as the oceanic trenches or subduction zones (regions of convergence overlying cold downwelling currents), mid-ocean ridges (spreading regions delineating upwelling), and hotspots (areas with anomalously high volcanism and heat flow indicating upwelling) are thought to reveal something of the nature of the underlying mantle circulation.

Two-dimensional models of mantle circulation, although useful for exploring the vertical structure and heat flow characteristics of convection (*1, 2*), cannot be used to address some of the major features of tectonics and convection, namely the horizontal geometry of the trenches, ridges, and hotspots. Studies of three-dimensional convection in a plane layer have indicated that various horizontal convective patterns involving both upwelling and downwelling plumes and sheets are possible (*3, 4*). However, the thickness of the earth's mantle is nearly half the radius of the entire planet, suggesting that spherical geometry should be used to obtain representative results. Studies of three-dimensional convection in a spherical geometry have focused on a shell that is entirely heated at its base (*5–7*), even though the earth's mantle has a large amount of internal heating from the decay of radioactive isotopes and secular cooling (*8*). We have used numerical models to examine the combined effects of various heating modes and spherical geometry on the three-dimensional structure of convection. We find that several aspects of mantle convection and plate tectonics are basic features of three-dimensional convective flow in a spherical shell.

Numerical models. The numerical models solve the three-dimensional fluid dynamical equations of mass, momentum, and energy conservation in a spherical shell having a ratio of inner to outer radii typical of the earth's whole mantle (approximately 0.55). (We do not address the issue of whole-layer versus two-layer convection; rather, we assume that whole-mantle convection is occurring.) The top and bottom boundaries of the shell are assumed to be impermeable and free slip because the earth's surface (with mobile plates) and the core-mantle boundary (with an underlying liquid core) are essentially surfaces of zero shear stress. The mantle is

D. Bercovici and G. Schubert are in the Department of Earth and Space Sciences, University of California, Los Angeles, CA 90024. G. A. Glatzmaier is with the Earth and Space Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.