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Phenotypic Characteristics of Neonatal T Cells

1. INTRODUCTION

T cells act as the "chef d'orchestre" in the adaptive immune response. They cooperate with B cells in antibody production, are effectors of the antigen-specific cell-mediated immunity (CMI) response, and exhibit regulatory properties that modulate the function of antigen-presenting cells (APCs), B cells, and various subsets of T cells. By virtue of secretion of cytokines, T cells also modulate the function of nonlymphoid somatic cells.

T cells are divided into various subsets based on the structure of their receptor, the expression of cytodifferentiation antigens, and their functions. The defining marker of the T cell is the T-cell receptor (TCR).

Two distinct subsets of T cells have been characterized based on the structure of the TCR: TCR-α/β and TCR-γ/δ.

Based on cytodifferentiation antigens, T cells were classified into two subsets: $CD4^+$ and $CD8^+$. $CD8^+$ T cells are effectors of CMI responses. They are able to lyse autologous cells infected with microbes, allogeneic cells, or tumor cells. They recognize peptides derived from endogenous proteins bound to major histocompatibility complex (MHC) class I molecules. $CD4^+$ T cells recognize peptides derived from the processing of foreign or self-antigens. They recognize these peptides in association with MHC class II molecules.

The $CD4^+$ T cells have been divided into three subsets based on their functions: Th1 cells, which play a role in antimicrobial defense reactions and release cytokines with multiple pleiotropic properties; Th2 cells, which cooperate with B cells in the production of antibodies and secrete cytokines that exhibit various regulatory properties; and regulatory T cells. The regulatory T cells are divided in other minute subsets based on their pattern of cytokine expression or on the expression of cytodifferentiation antigens. Regulatory T cells producing mainly transforming growth factor (TGF)-β are called Th3; those producing mainly interleukin (IL)-10 are called Tr1; and those exerting their effect by contact with effector cells exhibit a defined phenotype: $CD4^+$ $CD25^{high}$. Table 28 illustrates phenotypic characteristics of subsets of $CD4^+$ T cells.

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Table 28
Phenotypic Characteristics of Subsets of CD4 T Cells

Properties	Effector cells		Regulatory cells		
	Th1	Th2	Th3	Tr1	$CD25^{high}$
Cytokine production					
IL-2	+++	+	–	–	?
IL-3	++	+++	?	?	?
IL-4	–	+++	?	?	?
IL-5	–	+++	?	?	?
IL-6	+	+++	?	?	?
IL-10	+	+++	±	+++	?
IL-13	+	+++	?	?	?
IFN-γ	+++	–	?	?	?
TGF-β	+++	–	+++	±	?
Effect on B cells					
Activation of B cells	–	++	–	–	–
Class switching	IgG2a	IgG1, IgE	–	–	–
Autoimmunity					
Mediating autoimmune reaction	+	–	–	–	–
Preventing autoimmune reaction	–	++	+++	+++	+++

2. THE T-CELL ANTIGEN RECEPTOR: GENE REARRANGEMENT DURING THE ONTOGENY AND SIGNALING MECHANISMS

Like B cells, T cells are clonally distributed, because all the individuals of a clone share an identical TCR. The TCR is a heterodimer comprised of two chains (α and β or γ and δ), which are intimately associated with the CD3 complex. CD3 is comprised of five monomorphic polypeptides.

In humans, the TCR-α and -δ loci are localized on chromosome 14q11, the TCR-β locus on chromosome 7q35, and the TCR-γ locus on chromosome 7p15. The CD3 genes encoding γ, δ, and ε chains, which are structurally similar, are localized on chromosome11. CD3ζ 2 and CD3-ζ η are disulfide linked to CD3 and are important for signaling after the interaction of the TCR with MHC-peptide complex. The *TCR V* genes express clonotypic-idiotypic antigen markers, and some constant *TCR* genes express allotypic markers.

The TCR is encoded by three or four segments, which recombine during the development of T cells in the thymus. The organization of TCR-α and TCR(-γ genes is similar to that of genes encoding the immunoglobulin light chain, whereas the organization of TCR-β and TCR-δ genes is similar to that of genes encoding the heavy chain of immunoglobulin.

The TCR-α promoter located 5' to the V-α locus has two transcription sites: Pu, a purine-rich region, and GT, which is recognized by Sp transcription factors.

In humans, the TCR-α locus is composed of approx 50 V-α, more than 70 J-α gene segments, and one C-α. The enhancer is composed of four elements: (a) Tα1, which contains a DNA binding site for CRE;(b) Tα2, which contains sites for TCF-1 and Ets; (c) and Tα3, which contains sites for GATA, AP-2 and kE2 transcription factors. It is noteworthy that TCR V-δ locus is located between the TCR V-α and J-α loci. The transcription factors binding to $T\alpha^4$ are unknown.

The TCR V-γ locus is composed of 14 genes encoding variable chains (6 of which are pseudogenes) and two clusters of J-Cγ genes. The first cluster contains three Jγ1 and Cγ1 genes, and the second cluster contains two Jγ2 and one Cγ2 genes. The enhancer contains several segments: NFγ1, which binds GATA; NFγ2, which binds Ets; and NFγ3 and NFγ4, both of which bind CBF transcription factors.

The promoter of the TCR-β locus contains several potential binding sites for CP-1, Ap-1, poly designated as a polyoma virus enhancing element, two-conserved decamer, and one nonamer. In humans, there are about 60 *V*-β genes that can rearrange with one Dβ1, six J-β1, and one Cβ1 or with one Dβ2, seven Jβ2, and one Cβ2. V-β enhancer contains several regulatory regions.These include Tβ2, which binds GATA and Cre; Tβ3, which binds Ets; Tβ4, which binds CBF; and Tβ5, which can bind TCF/LEF-I transcription factors.

The TCR-δ locus is composed of three *V* genes encoding V-δ chains, which can recombine with three D-δ segments, three J-δ segments, and one C-δ segment. The enhancer of the TCR-δ/γ locus contains several segments that are able to bind transcription factors. Figure 37 illustrates the organization of human TCR loci.

The promoters of TCR-α and TCR-β loci are not lineage-specific and are active in various cells when coupled with various enhancers.

The rearrangement of various genes encoding TCRs follows the same rules as the rearrangement of gene segments encoding BCRs. This concept is supported by three groups of findings. First, the TCR-encoding genes have identical recombination signal sequences to those of V genes encoding the BCR. Second, TCR genes in embryonic conformation transfected into pre-B cells underwent rearrangements. Third, the immature T cells in the thymus express recombinant activating gene (RAG) enzymes. The rearrangement of genes encoding TCRs occurs in double-negative (DN) T cells in the thymus. RAG expression was found in immature $CD4^+$ $CD8^+$ $CD3^+$ T cells but not in the more mature single-positive (SP) thymic T cells. Thus, the coexpression of RAG1 and RAG2 in immature T cells in the cortical zone of the thymus mediates TCR rearrangement *(783)*. When productive rearrangement is achieved and the TCR-CD3 heterodimer is expressed on the surface, the expression of RAG may continue and allows for a secondary TCR-α recombination until the TCR is crosslinked during positive selection. RAG expression ceases in mature thymic T cells following TCR crosslinking and has never been detected in peripheral mature T cells in lymphoid organs.

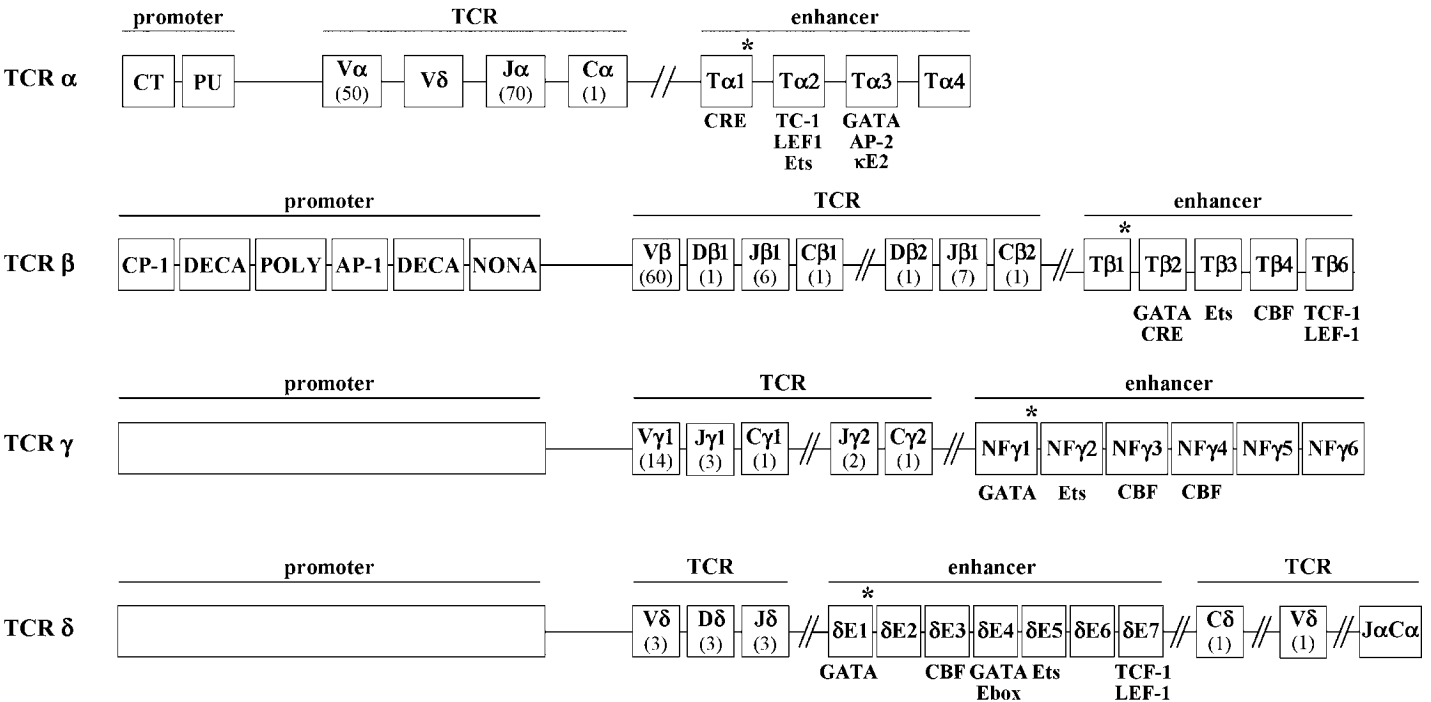

Fig. 37. Organization of human TCR loci.

In mice and humans, there is an order to the rearrangement of genes encoding TCR in the fetal thymus. In mice, the TCR-γ locus is transcriptionally active at d 14, reaches a peak at day 15, and rapidly decreases during gestation. The TCR-α and TCR-β loci are transcriptionally active by d 17 of gestation *(316)*. In humans, the TCR-δ locus is rearranged first, followed by TCR-γ, TCR-β and TCR-α loci *(336)*.

The expression of the TCR-CD3 complex is preceded by the expression of the pre-TCR complex in immature $CD25^+$ $CD4^-$ $CD8^-$ thymic cells. The pre-TCR was discovered in mice by von Bohemer and colleagues *(317)*. It is a heterodimer composed of pre-T-α, TCR-β, and CD3. The sequence of the pre-T-α gene revealed an opening reading frame of 618 nucleotides encoding a protein with a hydrophobic leader of 23 amino acids. The extracellular moiety consists of 130 residues, with two invariant cysteine residues that form interchain disulfide bonds like in Ig molecules. A third cysteine in position 119 could be used to form a disulfide bond between the pre-T-α and TCR-β chains. The extracellular domain has only 25% homology with TCR-α. The pre-T-α does not contain a J segment and ends at the Ig-like C domain. The intracytoplasmic region of 31 residues is proline-rich and contains an SH2 domain binding region and PPGHR motif also present in the tail of CD2, which is known to be involved in T-cell activation. The cytoplasmic tail of pre-T-α is longer than that of TCR-α and TCR-β chains. The pre-T-α–TCR-β complex is transported to the surface of immature T cells and is sufficient to promote T-cell development in the absence of other TCR chains. Intracellular signaling by the pre-TCR may be essential for the proliferation of immature thymic cells. During signaling via pre-TCR, the $p56^{lck}$ may be recruited by the pre-T-α protein into the pre-TCR complex *(317)*. In humans, the pre-TCR complex is expressed in the CD4 immature SP stage of differentiation of thymocytes and is downregulated in immature double-positive (DP) T cells in which all loci are rearranged. CD4 immature SP thymic cells are defined as pre-T cells, which have lost the CD34 marker of hematopoietic stem cells (HSCs) and express CD1a, CD5, and CD4 but not CD8. The rearrangement of TCR loci is temporally coordinated with the expression of CD44, CD25, CD4, and CD8 antigens.

Engagement of the TCR by its natural ligand, the MHC-peptide complex, triggers impressive signaling machinery in T cells *(784,785)*. As the CD3-TCR complex lacks intrinsic enzymatic activity, its involvement in signal transduction is mediated by the interaction with nonreceptor protein tyrosine kinases (PTKs). The TCR recruits various signaling molecules by the conserved sequences on the cytoplasmic tails of the CD3-TCR complex, immunoreceptor tyrosine-based activating motifs (ITAMs) *(786–790)*. There are 10 tyrosine-containing ITAMs on the CD3-TCR complex (six from the ζ-homodimer, two from ε chains, and one each from γ and δ chains) *(791,792)*. Depending on the

nature of TCR ligation, the tyrosine residues within the ITAMs can be differentially phosphorylated *(793)* and thus mediate the recruitment of particular signaling molecules, which in turn activate various biochemical cascades, leading to full T-cell activation, partial activation, or unresponsiveness *(794,795)*.

An early event following TCR ligation is the autophosphorylation of CD4-associated $p56^{lck}$ *(796–800)*, an Src-family kinase expressed exclusively in lymphoid cells and especially in T cells *(801,802)*. The T cells that are deficient in $p56^{lck}$ display a substantial reduction in TCR signaling capacity *(803,804)*. Animals deficient for this kinase exhibit a marked thymic atrophy associated with a significant decrease in the number of $CD4^+$ $CD8^+$, $CD4^+$ $CD8^-$, and $CD4^-$ $CD8^+$ thymocytes *(805)*. The $p56^{lck}$ kinase phosphorylates the ITAMs of CD3 components (γ, δ, and ε) and the TCR ζ-chain *(786–789)*, generating docking sites for the SH2 domain of various PTKs and protein adaptors involved in TCR signaling (ZAP-70, phospholipase C [PLC]γ1, SLP-76, Vav, c-Cbl, Shc, and TRIM) *(806–813)* and in CD28 signaling (PI-3K and PI-4K) *(814,815)*. ZAP-70 kinase of the Syk-family *(816,817)* tyrosine phosphorylates LAT *(818)*, which is an adaptor for Grb-2 and PI-3K. The PI-3K kinase controls the inositol lipid metabolism *(819)*. The stimulatory capacity of the TCR alone on PI-3K is weak, but the combined signaling of the TCR and CD28 yields the optimal serine phosphorylation of the p110 subunit of PI-3K *(820)*.

Ligation of the TCR triggers activation of several other PTK substrates, including CD5 *(821)*, Erzin cytoskeletal proteins *(822)* vasolin-containing protein *(823)*, and PLCγ1 *(824)*. Recruitment of PLCγ1 to the TCR mediates events that control inositol lipid metabolism by generating inositol polyphosphates and phospholipids. These metabolites allow the TCR to regulate the intracellular calcium levels and the serine/threonine kinase family of protein kinase C (PKC) isoenzymes. The TCR can also activate the guanidine nucleotide binding protein $p21^{ras}$ by a PTK-mediated mechanism independently of PLC-γ coupling to the TCR *(825)*. The $p21^{ras}$ component is critical for the cytokine-mediated proliferation of T cells and is mainly regulated by the Shc/Grb-2/Sos complex *(826)*. The TCR triggers this pathway only in certain populations of T cells *(809,827)*. In activated T cells, ligation of the TCR involves another Grb-2-like protein, the Crk-C3G protein complex used to catalyze the guanidine nucleotide exchange on $p21^{ras}$ *(828)*. Another PKC-sensitive Ras exchange protein that is activated by TCR signaling involved in the activation of T cells is the proto-oncogene Vav *(829)*, an exchange protein for Ras-related Rho/Rac proteins *(830)* that facilitates the intersection of TCR and CD28 signaling pathways *(831)*. The ERK1 and ERK2 of the mitogen-activated protein kinase (MAPK) cascade *(832)* mediate propagation of $p21^{ras}$ signals to the nuclear transcription factors. In T cells, there are two signaling pathways for ERK2 regulation: one that involves Ras and an-

other that involves PKC *(833)*. Activated $p21^{ras}$ but not PKC couples the TCR to the MAPK cascade *(834)*. Another major function of $p21^{ras}$ is to activate and recruit the serine/threonine kinase Raf-1 to the membrane *(835)*, which can trigger activation of ERK2. Lack of Raf-1 recruitment to the membrane was observed in some anergic T cells *(836)*. ERK2 kinase phosphorylates Elk1 *(837)*, a signaling molecule that associates in ternary complexes with a transcriptional activator serum factor that has roles in the regulation of Fos gene expression. Fos oncoproteins associate with the AP-1 transcription factor to induce activation of several cytokine genes. Two additional MAPK signaling cascades regulate JNK1 and JNK2 *(832)*. These two kinases integrate both TCR and CD28 signaling pathways with the Ca^{2+}/calmodulin system *(838)*. Regulation of JNK1 and JNK2 involves a kinase cascade similar to those of ERK1 and ERK 2. Ca^{2+}/calmodulin is another major signaling system activated by the TCR. The IP_3 inositol metabolite generated by PLC-γ binds to receptors in the endoplasmic reticulum to initiate release of intracellular Ca^{2+} stores. The rise of Ca^{2+} together with diacylglycerol (another PLC-γ-generated metabolite) activates PKC *(839)* and coincides with opening of CRAC channels that allow influx of extracellular Ca^{2+} into the cytoplasm *(840)*. Increased intracellular Ca^{2+} activates calcineurin (CaN), a ubiquitously expressed phosphatase with diverse functions*(825)*. CaN binds to NFAT transcription factors *(841)* and mediates its dephosphorylation-dependent translocation into the nucleus *(842,843)* in association with members of AP-1 family of transcriptional factors *(844,845)*. Increase of intracellular Ca^{2+} correlates with T-cell activation events *(846)*, whereas low- and noninducible calcium levels are characteristic for anergic T cells *(847,848)*.

These three major TCR signaling pathways (PKC, Ca^{2+}, and Ras) operate synergistically during the regulation of various transcriptional factors implicated in activation of the cytokine genes such as NFAT, NF-κB, and AP-1. Experiments involving dominant inhibitory mutants or specific inhibitors of these pathways suggest that the PKC system synergizes with the Ca^{2+} system in activating NFAT and AP-1. However, inhibition of PKC does not preclude activation of these transcriptional factors *(849)*. In contrast, the Ras signaling pathway is essential for the activation of NFAT *(850)* and can substitute for PKC signaling. A blockage of the Ras pathway, which is found in most of the anergic T-cell systems *(851)*, prevents the activation of ERK and JNK proteins *(852)* with subsequent downregulation of the AP-1 transcriptional activity. Activation of the Ca^{2+} signaling system can also suffice for transcription of the *IL-4* gene *(853)* but seems to be used mostly by the Th1 but not Th2 cells *(854)*. These observations revealed a branching nature of the TCR signaling machinery that is able to select particular biochemical pathways that ultimately lead to a specific T-cell effector function.

Tuning the TCR on T cells is the result of a fine balance between the PTKs and protein tyrosine phosphatases (PTPs).

3. EXPRESSION OF COSTIMULATORY RECEPTORS DURING THE DEVELOPMENT OF T CELLS

Numerous molecules are associated with the membrane of T cells (*see* Table 11). Some of these macromolecules function as coreceptors. Others represent markers of the T-cell lineage, activated T cells, or memory T cells.

3.1. CD4 and CD8 Coreceptors

CD4 and CD8 are markers that divide T cells into two main subpopulations. CD4 and CD8 display different functions and also function as coreceptors. CD4 is necessary for interaction with MHC class II molecules, which present peptides generated in the endosomal pathways, whereas CD8 interacts with MHC class I molecules, which present the peptides derived from the processing of endogenous proteins to the TCR.

CD8 is a heterodimer of 32 kDa made up of two chains, α and β, encoded by genes located on chromosome 2. CD8 binds to the α-3 domain of MHC class I molecules.

CD4 is a transmembrane glycoprotein of 55 kDa and is encoded by a single gene. The outermost domains of CD4 interact with MHC class II molecules, leaving the outer MHC domain that contains the peptide to interact with the TCR.

The interaction of the MHC class II-peptide complex with the TCR and CD4 is essential for the activation of T cells. This was clearly demonstrated by experiments in our laboratory. We prepared a chimeric, soluble, dimeric MHC class II molecule made up of a peptide that was derived from influenza virus hemagglutinin (HA) corresponding to110 to 120 amino acid residues, connected via a linker to the first exon of β chain of I-E^d and Fcγ2a. This chimeric molecule was coexpressed in a plasmid with the *I-E^d α-chain* gene, and after transfection, a dimeric soluble MHC class II-peptide chimeric molecule called DEF was obtained *(855)*. This soluble DEF molecule activated and induced the proliferation of T cells from transgenic (Tg) mice expressing the TCR that recognized HA110-120 peptide. The proliferation of HA-specific T cells was inhibited by both anticlonotypic and anti-CD4 monoclonal antibodies *(856)*. This finding indicates that the crosslinking of CD4 and TCR, and the resulting TCR-CD4 coaggregation, leads to stimulation of T cells as long as both TCR and CD4 molecules bind to the same ligand *(856)*.

Several observations suggest a functional integration of the CD4 and TCR signaling pathways in T cells: (a) CD4 associates physically with the TCR during T-cell activation *(857–859)*; (b) coaggregation of CD4 with CD3-TCR complex augments T-cell proliferation *(860)*; and (c) $p56^{lck}$ coimmunoprecipitates

with TCR phospho-ζ elements in activated T cells but not in resting or anergic T cells *(861)*.

The CD4 signaling pathways are not well defined. Several reports indicate that signaling of the CD4 coreceptor might affect the trend of TCR signaling cascades. Whereas some groups found that ligation of CD4 by antibodies induces T-cell activation independently of TCR signaling *(862)*, others found that anti-CD4 antibody induces production of tumor necrosis factor (TNF)-α and interferon (IFN)-γ but does not affect IL-2 or IL-4 production *(863)*, Ca^{2+} immobilization, tyrosine phosphorylation of Shc *(864,865)* or Ezrin protein *(866)*, or activation of $p59^{fyn}$ *(867)*, AP-1 *(862)*, and NFAT *(864,867)*. Discrepancies among these studies may reflect experimental differences such as the CD4 epitopes recognized by various antibodies, the degree of crosslinking, and the differentiation status of the T cells analyzed. In other experimental systems, ligation of CD4 by antibodies or HIV-1 gp120 independently of TCR-CD3 signaling induced a long-term anergy *(868–870)*. Also, lack of CD4-MHC class II-peptide interaction leads to T-cell anergy *(871)*.

The CD4 coreceptor plays an important role in T-cell differentiation *(872–874)*. Lack of CD4 on T cells has been associated with poor differentiation of DN T cells into Th1 or Th2 effector cells *(875)*. It has been suggested that oligomerization of CD4 molecules with the TCR-CD3 complex is an important step for CD4-mediated signal transduction in T cells *(876)* by virtue of the intracellular crosslinking of CD4 with the TCR-CD3 complex via CD4-$p56^{lck}$ kinase *(869–871,877,878)*. A Zn^{++}-based motif has been reported to mediate the noncovalent association of p56lck with the cytoplasmatic tail of CD4 antigen *(879,880)*.

3.2. CD28 and CTLA4 Coreceptors

Optimal activation of T cells requires two signals: one delivered by the TCR and another by CD28 superfamily coreceptors. According to the two-signal model, the lymphocytes fail to respond adequately in the absence of a CD28-induced signal.

The ligands of CD28 and CTLA-4 are CD80 and CD86, which are expressed on the surface of APCs.

CD28 is constitutively expressed on the surface of T cells, whereas CTLA-4 is rapidly expressed after the activation of T cells *(881)*. Binding of CD28 to CD80 and CD86 transmits a signal that synergizes with the TCR signal. The engagement of CD28 alone subsequent to exposure to anti-CD28 antibody does not activate the T cells in the absence of occupancy of the TCR by MHC-peptide complex. CD28 signaling regulates the threshold for T-cell activation and augments and sustains the T-cell response initiated by TCR signaling that favors T-cell proliferation, differentiation, and secretion of cytokines *(882)*. In

contrast, the engagement of CTLA-4 subsequent to interaction with CD80 and CD86 delivers a negative signal that inhibits the signals delivered by the TCR, CD28, and CD4 or CD8. The signals delivered via CTLA-4 inhibit the synthesis of IL-2 and the proliferation of T cells and terminate T-cell responses *(883,884)*. Therefore, the magnitude of the T-cell response involves a balance of CD28-mediated activation and CTLA-4-mediated inhibition after interaction with CD80 and CD86 ligands.

In mice, CD28 is expressed very early in ontogeny in an 11- to 12-d-old embryoid body derived from $CD45^+$ HSCs. In contrast, CTLA-4 was not found in embryonic cells or embryonic bodies by staining with specific antibodies, or its transcript was not detected by polymerase chain reaction *(416)*.

Study of CD28 and CTLA-4 expression in human neonatal lymphocytes showed that cord blood resting lymphocytes expressed higher levels of CD28 than adults. The proportion of $CD28^+$ T cells declines throughout life and was noted predominantly in the $CD8^+$ subset. Following in vitro activation with PMA and ionomycine, the percentage of $CD28^+$ adult T cells increased to a level similar to that seen in neonatal T cells. In neonatal T cells, CD28 is functional because signaling via CD28 enhances proliferation and synthesis of cytokines by murine neonatal T cells stimulated in vitro with mitogens or anti-CD3 antibody. Higher expression of CD28 in neonatal T cells may allow increased crosslinking to provide signals required for the activation/proliferation of T cells in peripheral lymphoid organs. In contrast, CTLA-4 was not expressed in resting neonatal T cells even after the stimulation in vitro with anti-CD3 antibody. Interestingly, CTLA-4 was detected in the cytoplasm of neonatal T cells. This suggests that the translocation of CTLA-4 on the membrane of neonatal T cells may be differentially regulated relative to adult T cells, in which CTLA-4 is expressed on the membrane after activation *(417)*. Because the activation of resting T cells depends on the balance of the expression of CD28 and CTLA-4, these observations suggest that high expression of CD28 in neonatal lymphocytes represents an important factor in T-cell activation.

3.3. CD40 Ligand (CD154)

CD40 ligand (CD40L) expressed on T cells interacts with CD40 expressed on B cells. This interaction leads to activation of B cells and plays a crucial role in class switching. In contradistinction with adult T cells, the expression of CD40L in neonatal T cells is reduced or undetectable *(885–887)*. Neonatal T cells were induced to express CD40L by stimulation with anti-CD3 antibody *(887)*, PMA, and ionomycine *(419,886)*. The kinetics of CD154 expression subsequent to activation with anti-CD3 antibody was similar in neonatal and adult T cells. The expression of CD154 on activated neonatal T cells may be

related to the observed differential regulation of CD154 transcripts by PMA and ionomycine *(419)*. The differences observed in the CD154 expression in young lymphocytes may be responsible for the predominant IgM secretion by B cells in postnatal life. The blocking of CD40L with CD40.G1 monoclonal antibodies, which bind to CD40L, prevented interaction with B-cell CD40 and inhibited the downstream switch reflected in inhibition of IgE and IgG4 antibody production *(887)*.

The expression of CD40L on T cells induced greater expression of CD86 on B cells *(887)* and also is able to induce the proliferation of thymic TCR-γ/δ cells and γ-mediated cytolytic activity and the secretion of IFN-γ and TNF-α *(888)*.

Thus, lack of expression of CD40L on neonatal T cells correlates with lack of class switching and the predominance of IgM production by neonatal B cells. However, T-cell activation by endogenous ligands leading to the expression of CD40L may favor class switching to IgE and IgG4 production during postnatal life.

3.4. CD2 (Lymphocyte Function-Associated Antigen [LFA]-2)

CD2 is expressed on all T cells. CD2 is an adhesion molecule of 55 kDa that interacts with CD48, CD58 (LFA-3), and CD59 expressed on different cells including APCs. The adhesion domain of human CD2 bears a single *N*-glycan at Asn65, which is required for adhesion to stabilize the polypeptide conformation *(889)*. The CD2–CD58 interaction, like anti-CD2 antibodies, induces the resting cells to be cycled and transduce an activating signal to the T cells. The interaction between CD2 and CD58 is facilitated by CD44, a glycoprotein of 80 kDa that functions as a homing receptor (reviewed in ref. *67*).

In humans, $CD34^+$ common lymphocyte progenitors enter the thymus at wk 7–8 of gestation. These precursors can differentiate into T cells and natural killer (NK) cells. They do not express CD4, CD8 CD3, or TCRs and are termed triple-negative (TN) T-cell precursors. By d 8.5 of gestation, these precursors express CD2 antigen, and a few days later, CD3 ε-chain can be detected in their cytoplasm. This observation indicates a temporal sequence of expression of CD2 before CD3 ε-chain in the early phase of gestation within a relatively narrow window of time. At this stage, the $CD2^+$ TN thymocytes can be stimulated to proliferate with anti-CD2 antibody and submitogenic amounts of IL-2 *(890)*.

Interaction of CD2 with CD58 increases cellular interaction and transduces activating signals to T cells. CD58 is expressed on thymic stromal cells. The binding of CD2 to endogenous ligand CD58 cannot trigger thymocyte activation alone but induces the expression of CD25 (IL-2R). Thus, CD2 expressed on a $CD34^+$ TN T cell likely does not induce activation signal as in mature T cells but rather upregulates the expression of IL-4R and the CD2–CD58 interaction. In concert with small amounts of IL-4, these effects may drive the proliferation and subsequent differentiation into $CD3^+$ DN T cells.

3.5. CD5

CD5 is a monomeric protein of 67 kDa that is expressed on T cells and a subset of B cells (B1). Its ligand is CD72, a C-type lectin expressed on B cells. CD5 has a long intracytoplasmic moiety, which is substrate for the tyrosine kinases *lck* and *fyn*. The intracellular fragment is in close association with CD3ζ-chain, and phosphorylation of CD5 occurs within seconds of binding the MHC-peptide complex to the TCR or after exposure to CD5 antibody. Stimulation via CD5 induces calcium mobilization, increase of cyclic guanosine monophosphate (cGMP), and the expression of CD25. Similarly to CD2, CD5 is expressed on $CD34^+$ TN immature thymocytes after wk 8–9 gestation.

3.6. CD34

CD34 is a cell-surface antigen of 120 kDa that is expressed on pluripotential HSCs and on the most primitive human T-cell precursors (TN thymocytes). $CD34^+$ TN thymocytes are present in the fetal thymus at wk 8.5 of gestation and differentiate into DN T cells. This concept is supported by several findings. First, thymic $CD45^+$ TN cells express the CD2 and CD5 antigens, which are expressed on all T cells *(891,892)*. Second, purified $CD45^+$ TN thymic cells dedifferentiate into DP T cells in fetal organic cultures using the fetal thymus from donors at wk 17–20 of gestation, whereas $CD34^-$ TN thymocytes lack this capacity *(893)*. $CD34^+$ TN thymic cells exhibit incomplete rearrangement of the TCR-δ locus displaying Dδ2–Dδ3 and Dδ2–Jδ2 rearrangements, whereas the TCR-γ, -β, and -α loci are in the embryonic germ line gene configuration.

In vitro culture of these cells with anti-CD2 antibody and recombinant IL (rIL)-4 for 2 d gives rise to TCR-δ^+ cells *(892)*.

The expression of CD34 decreases drastically during the thymic differentiation process at the stage of $CD4^+$ $CD8\alpha^+$ β^- and ceases at the stage of DP T cells. These findings strongly suggest that CD34 is a marker of the most primitive T-cell precursors committed toward differentiation into T cells.

3.7. CD21 Receptor

CD21 receptor is a transmembrane molecule of 145 kDa. Its ligands are the C3b/C3g and C3d fragments of human C3, and it also has a low affinity for IgE, CD23, and Epstein–Barr virus (EBV) virus (reviewed in ref. *894*). CD21 is expressed on all stages of differentiation of the T-cell lineage but at the highest density level in the most immature $CD34^+$ TN thymocytes. On transition from the $CD34^-$ $CD1^+$ $CD4^{low}$ $CD8^-$ stage to DN T cells, CD21 expression decreases, reaching the level of mature T cells. CD21 is shed from the membrane, and the levels of soluble CD21 increase with increasing gestation; however, reduced levels were detectable in cord blood *(894)*.

The function of the CD21 receptor during various stages of differentiation of the T-cell lineage is unclear *(895)*. One may speculate that it contributes to the selection of the T-cell repertoire by inhibiting recombination activating gene (RAG) activity and, therefore, TCR gene recombination. It is also thought to increase the susceptibility of immature T cells to EBV infection.

3.8. CD45

CD45 is present on all lymphocytes but exists in several isoforms that serve to distinguish various lymphocytes and are specifically expressed on various stages of T-cell development. The gene encoding CD45 is located on chromosome 1. Exons 4, 5, 6, and possibly 7 can be spliced in various ways, yielding eight isoforms that differ in extracellular domains. The CD45 is expressed in primitive T-cell precursors. CD45RA and CD45RB are expressed in naïve T cells, and CD45R0 interacting with CD22 is expressed in activated mature and memory T cells. In humans, the majority of T cells in cord blood express CD45RA, whereas those in adult peripheral blood express CD45R0.

CD45 can positively regulate the TCR signaling components by dephosphorylation of negative regulatory tyrosines on PTKs *(896,897)* such as Tyr505 of $p56^{lck}$ *(898,899)*. Antigen stimulation in the absence of CD45 prevents T-cell proliferation and production of cytokines via hyperphosphorylation-mediated inactivation of $p56^{lck}$ *(900,901)*. In contrast, antibody-mediated coaggregation of CD45 with the TCR can negatively regulate the TCR signaling machinery *(902)*, presumably by CD45 interference with TCR oligomerization-mediated signaling *(903)* or by excessive dephosphorylation of the positive regulatory tyrosines such as Tyr394 or $p56^{lck}$ *(904)*. In general, the PTPs induce negative regulation of TCR signaling by dephosphorylation-mediated deactivation of PTKs. However, negative regulation of TCR signaling can be mediated not only by PTPs but also by some PTKs such as CsK tyrosine kinase *(905)*, which mediates phosphorylation of the inhibitory tyrosines of Src kinases (i.e., Tyr505 or $p56^{lck}$) *(906,907)*.

4. DEVELOPMENT OF THE T-CELL LINEAGE

During ontogeny, the development of T cells has been divided into three phases: the prethymic phase (which begins in the liver during gestation and continues in bone marrow during infancy and thereafter), the thymic phase, and the mature phase.

4.1. Prethymic Phase

In mice, the prethymic phase begins by d 14 of gestation in the fetal liver and continues in bone marrow during infancy and thereafter. Pluripotential HSCs present in the fetal liver or bone marrow differentiate into common lymphoid precursors and, thereafter, into T-cell precursors. Pluripotential HSCs

express markers of both myeloid and lymphoid lineages such as Mac-1, Gr-1, Thy1, B220, Tae, 11, c-Kit, and Scal-1 *(156)*. From pluripotential HSCs arise bipotent T/NK-cell precursors identified as $CD117^+$, $CD44^+$, and $CD25^-$. The T/NK precursors reach the thymus through blood, enter into the cortical medullary junction by passing between endothelial cells of capillaries, and then migrate in the cortical zone *(908)*. Although the bone marrow HSCs and pluripotential HSCs exhibit the properties of fetal HSCs, they are phenotypically distinct because they express other markers, specifically $Thy1.1^-$, $c\text{-}Kit^{bright}$, and Rho^{high} *(909)*.

4.2. Thymic Phase

Upon entry into the thymus, bipotent T/NK precursors differentiate in the thymic cortex. This process is not autonomous and requires signals delivered after contact with various types of mesenchymal and thymic epithelial cells (TECs). The differentiation process of T-cell precursors is characterized by temporally coordinated expression of cell surface markers, the expression of RAG and TdT enzymes, and the rearrangement of genes encoding TCRs. The T-cell progenitors then enter into the thymus at the cortical medullary junction and migrate into the cortex. The most immature stages are characterized by the absence of the expression of CD4 and CD8 markers and are termed as DN.

In mice, DN cells undergo four major stages of differentiation. The phenotype of DN1 and DN2 thymocytes is $CD44^{++}$ $CD25^-$, and $CD44^+$ $CD25^{++}$, respectively. Both DN1 and DN2 thymocytes synthesize the pre-T-α-chain. The phenotype of DN3 cells is $CD44^-$ $CD25^+$, and these cells synthesize the TCR-β-chain. DN4 thymocytes do not express CD44; they express CD25 and pre-TCR encoded by pre-T-α and TCR-β. There is a subtle subset of DN4 in which no intracytoplasmic TCR-β protein has been detected. In that TCR-β^- subset, intracytoplasmic TCR-γ/δ was detected *(910)*. These cells may exhibit a low level of proliferation and may differentiate into TCR-γ/δ^+ cells in the cortex or medulla. Accompanying this maturation is a second round of expansion, which corresponds to the transition of DN3 toward DN4 thymocytes. The proliferation is mediated by pre-TCR, because the differentiation is arrested at the DN3 stage in pre-TCR knockout mice. In this mouse strain, no proliferation of DN3 thymocytes occurs *(911)*. DN3 and DN4 thymocytes express a newly characterized antigen, CD147 *(912)*. CD147 may play a role in cell cycling, because the fetal thymic organ cultures using thymuses removed at d 14–15 of gestation showed no DP thymocytes after incubation with the anti-CD147 antibody. These cultures showed a relative increase of DN1 and a drastic decrease of DN4 cells. This finding suggests a potential role of CD147 in the second phase of expansion of immature thymocytes.

In all differentiation stages, the DN thymocytes express CD2 and CD5 antigens, and the RAG enzymes are active. DN4 thymocytes differentiate into DP cells that express CD4, CD8, and TCRs. They lose the expression of CD44 and CD25, and RAG activity is absent or reduced to low levels sufficient for additional rearrangement of TCR-α genes (TCR editing).

DP T cells differentiate into SP T cells that express CD4 or CD8 and T cells that express TCR-γ/δ or TCR-α/β.

In humans, the pathway of T-cell development is similar to that described in mice, with the exception of the expression of different antigens. The T/NK-cell precursors are characterized by $CD34^+$, $CD1a^+$, and $CD5^+$. The DN cells display three phases of differentiation according to the model proposed by Blom et al. *(336)*. From pre-T cells $CD34^+$, $CD1a^+$, and $CD5^+$ arise the preTCD4 immature SPs $CD34^-$, $CD1a^+$, $CD5^+$, $CD4^+$, and $CD8^-$. From these cells arise other types of DN cells called the EDP cells $CD34^-$, $CD1a^+$, $CD5^+$, $CD4^+$, and the CD8-α-chain. These cells differentiate into DP T cells exhibiting the phenotypes $CD1a^+$ $CD5^+$, $CD4^+$ CD8-α and -β^+ $CD27^-$, $CD69^-$, and TCR^+.

In the cortex, the majority (70%) of DP cells are not dividing cells; however, a small number (15%) are dividing cells that probably result from positive selection of DP cells. DP and SP T cells migrate from the cortex into the medulla. Kinetic analysis of DP and SP thymocytes suggests that the turnover time of DP and SP thymocytes in the medulla is significantly longer than that of DP in the cortex *(913)*. Once in the medulla, SP thymocytes are activated and express homing receptors. In the medulla, only 2–10.5 % divide, as assessed by a short-pulse labeling with BrdUrd *(913)*. In the medulla, SP T cells express HSA but not Qa2 antigen. The overall turnover time for medullar SP thymocytes is far greater than 1–2 d as was thought initially; it takes at least 6–9 d *(913)*. After residing in the medulla for several days, the SP T cells are exported to the periphery through veins and lymphatics. Their entrance into the vessels depends on expression of homing receptors, which are probably induced by cytokines in the medulla. Only the most mature SP thymocytes, which are HSA^-Qa2^+, leave the thymus.

The pathway of the differentiation process of murine and human T cells is illustrated in Fig. 38.

5. POSITIVE AND NEGATIVE SELECTION

Stochastic recombination of the gene segments encoding TCRs potentially create specificity for all peptides derived from the processing of foreign and self-antigens presented to T cells in association with MHC class I and II and CD1 molecules. The specificity of the T-cell repertoire is established during fetal development of the thymus.

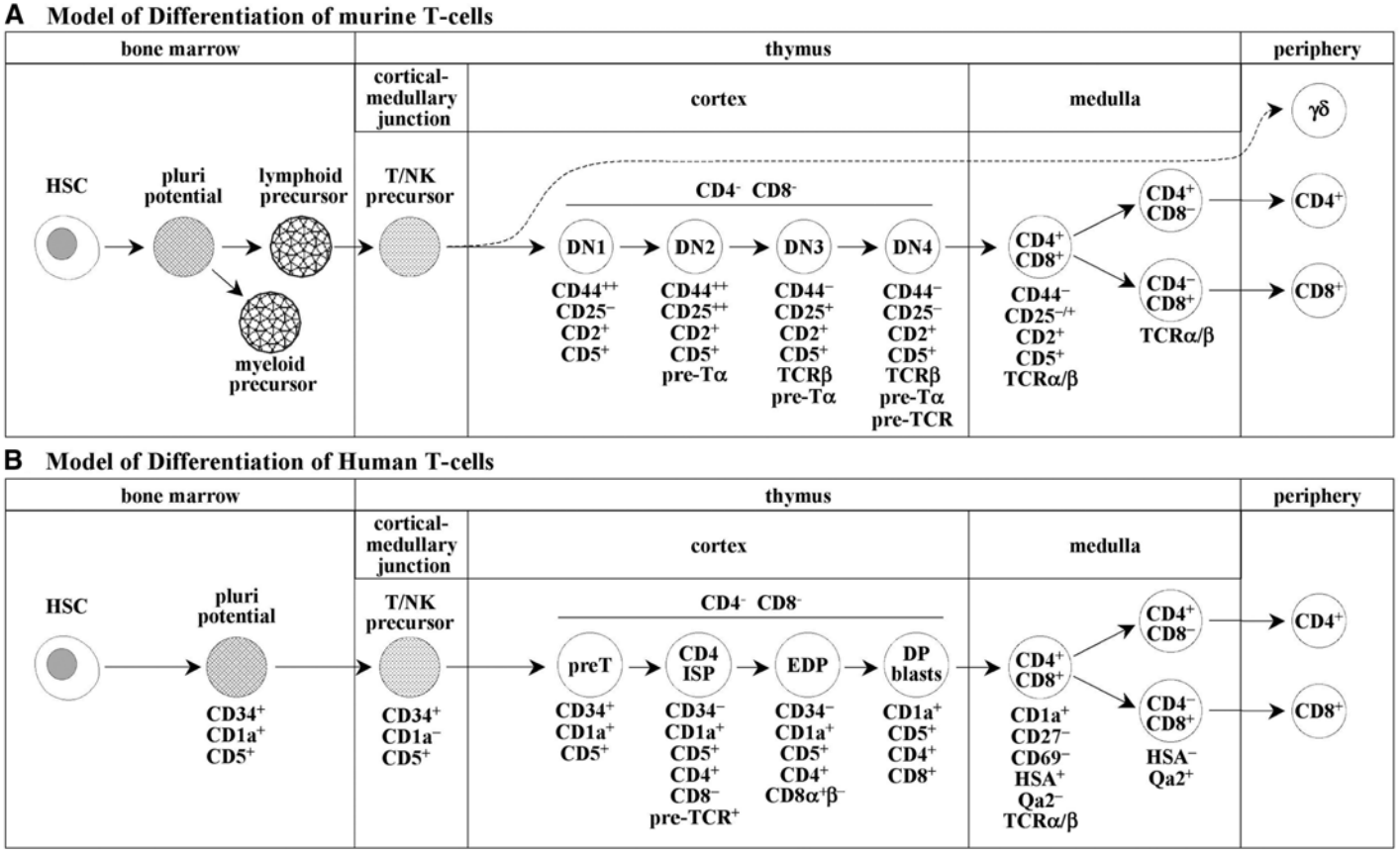

Fig. 38. Model of differentiation of murine and human T-cell lineage.

The major property of the immune system, including the T cells, is to discriminate between self and nonself. This implies a selection of the lymphocytes. T cells mediating the immune response against foreign antigens are positively selected, whereas a negative selection process deletes those that recognize self-antigen, which may cause autoimmune reactions.

Only T cells with a low affinity for MHC-peptide complex are positively selected, proliferate, and differentiate, whereas those exhibiting a TCR with high affinity for ligands are eliminated. This concept creates a paradox in which the signal induced by the ligand through the TCR results either in survival or death.

Positive selection is a multistage process involving interaction of T cells with the thymic environment and, in particular, with TECs expressing MHC molecules. TECs provide specialized accessory interactions that MHC^+ epithelial cells from other tissues do not. TECs and thymic stromal cells express other molecules such as BPM and TTS24, which could be essential in the positive selection process.

The most important question regarding the process of positive selection is: How does positive selection occur subsequent to interaction of TECs with thymocytes during fetal life, when the embryo is protected from exposure to foreign antigen that, with rare exceptions, cannot pass through placenta?

The paradigm of the positive selection process is that only cells that bear a TCR with low affinity/avidity for the self-derived MHC-peptide complex are positively selected. The positive selection process occurs in the thymus cortex at the most immature stage of differentiation of DP, large, dividing blast thymocytes that express a fully functional TCR and CD69, CD2, CD5, and Qa2 surface markers. Positively selected DP blast cells become small nondividing thymocytes located in the cortex. The interaction of the TCR with MHC-peptide complex is crucial for positive selection. This concept was supported by the study of TCRs in MHC knockout mice in which the differentiation of DP thymocytes was arrested (reviewed in ref. *914*). Positively selected DP thymocytes exhibit a new wave of expansion that appears to be MHC-independent and gives rise to SP thymocytes.

Positive selection is driven by binding the TCR to a single or mixture of self-peptides associated with MHC molecules on the surface of TECs. It is noteworthy that a few peptides derived from self-antigens have been identified. The majority of the peptides identified were not stimulatory for mature T cells, which require high-affinity interactions. Several transcription factors are critical for positive selection, such as Src and Syk family kinase proximally associated with the TCR, Ras, RasGRP, PLC-γ, ERK, helix–loop–helix family, Schnurri, and Egr1. The involvement of these factors in positive selection is supported by studies carried out in mice with targeted mutations in genes

encoding these factors. In these mice, positive selection and the DN to DP transition processes are blocked (reviewed in ref. *915*).

Negative selection results from the high-affinity interaction of self-peptides with TCRs. This concept is supported by several findings demonstrating T-cell deletion by increased apoptosis. Pertinent findings include (a) deletion of T cells induced by endogenous antigens and by injection of high amounts of peptides in TCR Tg mice, (b) the increased expression of peptides in the thymuses of Tg mice, and (c) massive apoptosis caused by injection of anti-CD3 antibody.

Clonal deletion occurs early in T cell development at the stage of transition of DN cells to DP cells but also occurs late in the SP stage. In addition to TCR–MHC-peptide complex high-affinity interaction, the negative selection requires a second costimulatory signal delivered by CD28–B7 or CD40L–CD40 interactions. This concept is supported by the observations that CD28 knockout mice are resistant to anti-CD3 antibody or peptide-induced apoptosis *(916)* and that CD40-deficient mice do not exhibit the negative selection induced by endogenous super antigens *(917)*.

It was generally considered that the negative selection clonal deletion results from activation of apoptosis mediated by Fas ligand–Fas receptor and TNF-α–TNFR interactions. Most studies derived from mice with targeted genes for FasR, TNFR1, and TNFR2 revealed that FasL or TNF-α are not necessary for negative selection. Similarly, in the case of selection induced by endogenous peptides, it was shown that TNF signaling is dispensable for negative selection *(918)*. More recent evidence suggests that orphan steroid receptor factor Nur77, a downstream regulator of TCR involved in the apoptosis pathway, increases negative selection *(919,920)*. Studies in knockout mice suggest that Grb2 (an adaptor protein associated with the TCR) signaling is important for negative selection *(921)*. Also, p38, which is an activator of c-Jun, and MKK7, which is an upstream activator of JNK, increase negative selection. Clonal deletion requires both RNA and protein synthesis. These observations strongly demonstrate that negative T-cell selection is an active process.

6. TCR EDITING DURING T-CELL DEVELOPMENT

The process of BCR editing was first discovered in the case of immature B cells. There are some observations that the same process is active during the T-cell development. In the case of T cells, the editing process results from rearrangement of the TCR-α locus, which continues in the cortex DP cells and even in the medulla SP T cells. In one model, a rearranged *V*α-*J*α gene was introduced (knock-in) in embryonic stem cells and expressed in DP thymocytes with the *TCR*-β gene. Editing in these mice was induced by a superantigen and was associated with the internalization of one TCR-α *(922)*.

TCR editing also was described in a Tg mouse strain in which ovalbumin was expressed under the control of the human keratin 14 promoter, which

drives the gene expression in TECs and skin, and tongue and esophagus keratinocytes but not in other tissues. In contrast to Ot-1 Tg mice that display a drastic reduction of DP thymocytes, in the K-14 OVA double-Tg mice, a significant proportion of DP thymocyte remained. These cells remained in the thymus because they expressed endogenous TCR-α; allelic exclusion of the TCR-α locus is not complete in mice. The DP thymocytes in these mice exhibited low expression of TCRs, CD4, and CD8 and an increased expression of CD69, indicating that they had encountered the antigen. Low expression of TCRs indicates that the autoreactive TCRs were internalized and that RAG expression was maintained in DP thymocytes of these mice. From these results, it was predicted that of the 10 to 27% of peripheral T cells expressing two functional *TCR*-α genes, some can express an autoreactive TCR *(923)*.

Taken together, these observations indicate that self-antigen presented by TECs can cause in DP thymocytes the internalization of TCRs and rearrangement of endogenous TCR-α, allowing for survival of DP T cells by a receptor editing process. Because these conclusions derive from studies carried out on Tg mice, it is difficult to determine the significance of the TCR editing process in vivo.

7. DEVELOPMENT OF T CELLS IN VERTEBRATES

CMI in T cells represents an important arm of defense reactions in all vertebrates. The thymus and T cells occurred 500 million yr ago, when jawed vertebrates diverged from other vertebrates. T-cell differentiation takes place in the thymus in all vertebrates. In all vertebrates, the TCR is the major marker of T cells, and the rearrangement of genes encoding the specificity of the TCR follow the same rules.

7.1. Ontogeny of T Cells in Fish

In all fish, T cells differentiate and mature within the thymus, which derives from the endoderm and the mesenchyme of pharyngeal pouches and the ectoderm of branchial clefts. The thymus anlage is the first lymphoid tissue that develops during ontogeny.

In fish, the pronephros is the organ that contains HSCs. From pronephros, the HSCs migrate into the thymus before the spleen.

Hansen and Zapata *(320)* divided thymic lymphopoiesis into four stages: (a) the occurrence of thymic primordium, (b) its colonization with precursors, (c) expansion and differentiation, and (d) the histological division of thymic tissue into the cortex and medulla.

In trout embryos, the thymic primordium is evident at 8 d prehatching. At hatching, the thymus consists of few layers of ETC, and at 1 d prehatching, lymphocytes become preponderant. One month after hatching the thymus is completely regionalized in the cortex and medullar areas. RAG activity was detected at d 10 postfertilization when neither pronephros nor thymus primor-

dia were developed *(924,925)*. These findings suggest that thymic progenitors may rearrange the genes encoding the TCR before colonization of the thymus. V[D]J recombination was also described in the thymus anlage at d 15 postfertilization. At d 16–20 postfertilization, the rearranged TCR-βV2 do not exhibit N insertion despite the fact that TdT is expressed before hatching.

In catfish, the thymocyte population changes remarkably during the first 28 d posthatching. At d 1, the thymus consists of epithelial cells and only a few lymphocytes. By d 5, the number of thymocytes increases and by d 21 occurs in equal proportions throughout thymic parenchyma *(253)*.

In zebrafish, the formation of the thymic primordium occurs much faster: 60 h postfertilization. At 65 h, the thymus anlage contains small and large lymphocytes distributed throughout the meshwork of ETC, and at d 4 postfertilization the number of thymocytes increases considerably. RAG expression coincides with colonization of the thymus with lymphocytes at 65–70 h postfertilization *(926)*.

7.2. Development of T Cells in Amphibians

In Xenopus, the thymus primordia become evident at d 3 postfertilization and 1 d later are colonized with lymphocytes. The colonization of the thymus with precursors is characterized by several waves. The first wave takes place by d 4 postfertilization by progenitors from embryonic mesoderm. The second wave results from colonization of the thymus by precursors originating from dorsal lateral plates. Finally, the third wave, which takes place during metamorphosis, results from seeding of the thymus with precursors from the larval liver (reviewed in ref. *320*). After the first wave of entrance of progenitors, 20% of thymocytes express the T-cell-specific XTLA-1 antigen. The first CD8 antigen appears at d 12 postfertilization. By this time, the larval thymus exhibits clear demarcation of the cortex and medulla. Following the expression of XTLA-1 and CD8 markers, the vast majority of thymocytes after the second wave of colonization express the CD5 marker of T cells *(927)*. RAG expression was observed d 3.5 postfertilization, and 1 d later the transcripts of fully rearranged TCR loci were detected.

Later during metamorphosis, over 90% of thymocytes in the cortical area are deleted. This observation strongly suggests that there is a negative selection in frog thymuses.

7.3. T-Cell Lymphopoiesis in Birds

The TCR and major markers of T cells defined in mammals appear to be highly conserved in birds. Bird T cells express TCR-γ/δ and TCR-α/β, a pan-T-cell antigen recognized by the CT1 monoclonal antibody, and CD2, CD5, CD4, and CD8 *(928)*. Similarly to lower vertebrate species, in birds the thymus is

seeded with precursors from blood in waves. Studies of T-cell development in the chick–quail chimera indicated that the thymus is the only site in which T cells expressing the TCR-CD3 complex are generated *(929)*. During T-cell development, the CD3 molecules were identified at d 9 of embryo development, and at d 11 about 20% of thymocytes expressed CD2 and then CT1 antigen. In the thymus there is a sequential differentiation of TCR-γ/δ and TCR-α/β subsets *(930)*. The thymocytes expressing TCR-γ/δ occur in the thymic cortex on d 12 of embryonic life, approx 5 d after HSCs enter the thymus and 3 d after the detection of CD3 molecules. By day 13 TCRs-γ/δ reach the medulla and their number increases, indicating that they enter into the cell cycle and proliferate. Intracytoplasmic TCR-β-chain was detected on d 12, and surface expression of the TCR-α/β-CD3 complex was detected in the cortex at d 14. The TCR-α/β subset quickly becomes predominant. The sequential development of thymocytes expressing TCR-γ/δ followed by those expressing TCR-α/β strongly suggests that in birds, these cells represent two different lineages. This concept is supported by an experiment that showed that the treatment of embryos with anti-TCR-δ monoclonal antibody prevented the development of TCR-γ/δ thymocytes without affecting the development of thymocytes expressing TCR-α/β. Similarly, treatment with anti-TCR-α/β antibodies prevented the development of TCR-α/β but not of TCR-γ/δ thymocytes *(931)*.

TCR-α/β thymocytes are DP and differentiate into SP T cells. They leave the thymus and occur in the spleen by day 19 and in the intestine by d 20 of embryonic life. It interesting that in birds, the majority of SP T cells in the periphery do not express CD2 *(928)*.

7.4. T-Cell Development in Lambs

As in other mammalian species, in lambs T lymphopoiesis takes place only in the thymus during the last trimester of pregnancy. Thymocytes and peripheral T cells expressing TCR-γ/δ occur before TCR-α/β. In the fetus during the last month of gestation, TCR-γ/δ represents 18% of total T cells, and their number increases in the cord blood to 60% *(932)*. Postnatal SP T-cell emigrants express CD11a/CD18, CD44, CD2, and CD58 antigens. Prior to export from the thymus, CD44 is upregulated on TCR-γ/δ cells, and both CD16 and CD58 are downregulated, suggesting that this process may be the final step of maturation of TCR-γ/δ cells. In contrast, there is a continuous upregulation of CD2 during thymic development as well as an upregulation of the expression of CD2 on both TCR-γ/δ and TCR-α/β T cells during postnatal life. Thus, it appears that the changes in the expression of costimulatory and adhesion molecules are the result of the maturation of thymocytes to a mature peripheral T cell *(933)*. The lifespan of thymocytes in the fetus is unknown. However, before parturition the fetal lamb develops a large pool of long-lived circulating T

cells that is rapidly replaced by short-lived T cells during postnatal life, following exposure to environmental antigens *(934)*.

7.5. Thymopoiesis in Pigs

In contrast to other mammalian species, the differentiation of T-cell lineage in pigs exhibits some particularities *(329)*.

The first lymphocytes occur in the thymic rudiment at d 38 of gestation. The vast majority are DNT cells expressing the pan-lymphocytic marker CD45 and the promyelocyte-monocyte antigen SWC3a. Early T-cell progenitors (CD3-ε^-, $CD4^-$, and $CD8^-$) have been identified only in the $SWC3a^-$ population. From these cells arise immature thymocytes characterized by the expression of CD8 and the absence of CD3-ε and CD4 expression. These cells differentiate in DP CD3-ε^- thymocytes, which at day 40 express CD3-ε^{high} and CD25. The DP thymocytes have been identified in the fetal thymus at d 56–76 of gestation. All DP thymocytes are positive for CD5, CD2, and CD1 expression *(935)*.

Thymocytes first differentiate into TCR-γ/δ CD3-ε^{high}. Until d 50 of gestation, TCR-δ thymocytes represent almost exclusively all $CD3^{high}$ thymocytes. The number of TCR-δ thymocytes decreases, and by day 55 TCR-β cells become predominant. Thus, like in other mammalian species, the TCR-δ T-cell subset occurs earlier than the TCR-α/β subset *(935)*.

Apparently, in pigs the thymus is colonized by at least two waves of HSCs; however, the influx of T-cell progenitors is discontinuous and decreases sharply between d 40 and 45 of gestation, when the frequency of $CD45^{high}$ thymocytes increases significantly.

7.6. Ontogeny of Human T Cells

In humans, the thymus develops from the third branchial pouch at wk 6 of gestation. Originally, the majority are ETCs and the lymphocytes begin to appear at wk 8 to 9 of gestation. Gradually the thymus separates into the cortex and medulla, and eventually Hassall's corpuscules are evident, marking the full development of the thymus gland. In thymuses from fetuses at wk 15 to 26 of gestation, 50 to 96% of thymocytes express CD2 as assessed by the ability to rosette sheep erythrocytes *(330)*. The immature thymic cells develop from $CD34^+$ $CD38^-$ fetal liver cells and, after birth, from CD 34^+, $CD10^+$ Lin^- c-Kit^- human bone marrow cells *(894)*. From these, HSCs derive T/NK precursors, which differentiate into DN, DP, and then SP thymocytes expressing TCR-α/β. The TCR-α/β cells develop exclusively in the thymus.

By contrast, in humans TCR-γ/δ T cells can be identified before thymic development at wk 6–8 of gestation in fetal liver cells. These cells also have been identified in cord blood cells and expand in vitro vigorously upon culture for 14 d with rIL-2.

In conclusion, in all vertebrate species the T cells develop within the thymus with the exception of TCR-γ/δ cells, which may develop extrathymically. Development of T cells expressing TCR-γ/δ precedes the occurrence of TCR-α/β cells. During fetal life, the HSCs are harbored within fetal liver. The common lymphocyte progenitor and T/NK precursors are derived from HSCs. During postnatal life and thereafter HSCs are present within bone marrow. The T/NK precursor cells later enter into the thymus at the cortical medullary junction, from which they migrate into the cortex. There, they undergo various stages of differentiation (e.g., DN to DP to SP). In these stages of differentiation, they first express a pre-TCR, and at the stage of DP they express fully functional TCR-α/β.

8. FUNCTION OF NEONATAL T CELLS

Since the original observation of Billingham and Medawar *(607)* that mice neonates injected with allogeneic bone marrow cells were able to accept skin allogeneic graft, it has been believed that the poor CMI response in neonates is related to immaturity of T-cell function and higher susceptibility to tolerance. However, tolerance by itself represents an active immune response because tolerant lymphocytes transferred into naïve mice conferred unresponsiveness to recipients. The concept of an active tolerance is supported by an experiment by Goldman et al. *(936)* showing that the activation of Th2 cells plays a role in the induction and maintenance of neonatal tolerance.

From a functional point of view, T cells are classified in three main subsets: (a) CD4 T cells, which were initially considered helper T cells; (b) CD8 T cells, which mediate cytotoxic reactions; and (c) regulatory T cells. CD4 T cells were divided in two additional functionally distinct subsets: (a) Th1 CD4 cells that produce IFN-γ and IL-2, which favor class switching to IgG2 and mediate the delayed hypersensitivity reaction and protection against intracellular microbes, and (b) Th2 CD4 cells that produce IL-4, IL-13, and IL-5, which participate in humoral responses and favor class switching to IgG1 and IgE.

Late studies from the 1970s that were carried out in mice challenged the concept of unresponsiveness of neonatal T cells. At first glance these studies were very crude in light of the progress made in T-cell immunobiology. However, these studies merit mention because they demonstrated that neonatal T cells are functional. Bosing-Schneider *(937)* measured the ability of neonatal T cells to secrete T-cell replacing factor (TRF), which is probably a mixture of cytokines secreted by Th2 cells. The study demonstrated that T cells from 1- to 2-d-old mice produce minute amounts of TRF; however, 1-wk-old mice had a sufficient number of T cells to produce as much of this factor as T cells from an adult. It was later shown that neonatal thymic cells are able to provide assistance to B cells producing IgM antibodies to TNP-BGG *(938)*.

Further studies carried out in mice demonstrated that neonatal CD4 T cells are functional and that Th2 responses dominate in neonates. Th1 responses are prevalent in adults. In vitro studies showed that although the frequency of neonatal murine Th2 cells in lymph nodes is similar to that observed in adult mice, virtually no IFN-γ-secreting Th1 cells were detected in neonatal spleens. The production of IL-4 by neonatal splenic Th2 was antigen-specific *(939)*. Thus, the neonatal naïve T cells are biased to secretion of IL-4, a Th2 cytokine, both under standard in vitro activation conditions *(939)* and after in vivo immunization of neonates with peptide delivered on an immunoglobulin platform that enhances the immunogenicity and provides adjuvant activity *(940,941)*.

Neonatal T cells rapidly lose the Th2 phenotype. By d 6 after birth, high production of IL-4 diminishes *(942,943)*. Nonetheless, the Th1 cells are also present in newborn lymphoid organs. This concept is supported by the following findings:

a. Upon in vitro culture of neonatal splenic cells in the presence of IL-6 or anti-CD28 antibodies, IL-2 production and proliferation of Th1 cells were enhanced, reaching the levels of adult T cells *(944)*.
b. In vivo administration of IL-12, a strong promoter of Th1 cell development, together with anergic cells induced the production of high levels of IFN-γ and IL-2, and these mice acquired the capacity to prevent the induction of neonatal tolerance *(945)*.
c. In vivo injection of anti-CD40 antibodies in neonates enhanced the alloantigen-specific Th1 response and prevented the induction of neonatal tolerance *(946)*.
d. Selective generation of neonatal Th1 responses were described with different modes of immunization such as immunization with hen egg lysozyme and Freund's incomplete adjuvant (FIA) *(947)* or subsequent to immunization with DNA vaccines. A robust Th1 response was described in newborn mice immunized with a plasmid containing influenza virus HA gene, as assessed by production of IFN-γ and IgG2a antibodies *(643)* or plasmids containing measles virus HA, C fragment of tetanus toxin, or Sendai virus nucleoprotein *(644)*.
e. Autoreactive Th1 cells develop soon after birth. This was clearly shown in two different animal models of autoimmune diseases. One model of juvenile insulin-dependent diabetes mellitus (IDDM) consists of double-Tg mice expressing influenza virus HA under rat insulin promoter in pancreatic β cells and a TCR specific for a dominant epitope of HA recognized by T cells in association with I-E^d MHC molecules. Five-day-old double-Tg mice developed periinsulitis, which coincided with an increased number of HA-specific diabetogenic T cells *(948)*. In a model for autoimmune ovarian disease (AOD), it was shown that immunization of females at d 2 and 5 after birth with pZP3 antigen in FIA led to development of AOD *(949)*. These observations clearly demonstrate that autoreactive Th1 cells, which mediate both IDDM and AOD, escape from tolerance and are present and functional after birth.

Under certain conditions, neonatal T cells mount a mixed Th1/Th2 response. This conclusion was strongly supported by an experiment in which mice immunized at birth with protein antigen *(950)* or DNA vaccines *(643,648,951)*

were able to mount quantitatively and qualitatively indistinguishable mixed Th1/Th2 responses 1 wk after immunization.

Both in vivo and in vitro experiments demonstrate that exposure to antigen usually gives rise to a Th2-biased secondary response *(648,947)*. It is unclear whether the Th2 dominance resulted from lack of priming or from recall of neonatal Th1 cells. This question was particularly important because it was demonstrated that both neonatal Th1 and Th2 cells were able to mount a primary response *(648,950,951)*.

Recently, Li et al. *(952)* attempted to address this question using a model in which neonatal T cells from a Tg mouse strain expressing a TCR specific for an ovalbumin-derived peptide were transferred into normal newborn mice. In this system, priming with Ig-Ova induced both Th1 and Th2 responses; however, the recall with antigen induced only a Th2 response. Surprisingly, the Th1 response after recall was observed when IL-4 was neutralized by exogenous anti-IL-4 antibody. In addition, anti-IL-4 antibody prevented in vitro antigen-induced apoptosis of Th1, indicating that IL-4 and IL-13 may be involved in IL-4-mediated targeting of Th1 cells in vivo following the challenge with ovalbumin-derived peptide. Thus, it appears that IL-4 may exert its downregulatory effect on the secondary response of Th1, because IL-4R and IL-13R proteins are expressed on surface of Th1 cells *(952)*.

An apoptotic effect of IL-4 on neonatal Th1 cells is supported by results of microarray analysis of genes encoding antiapoptotic and proapoptotic factors. Neonatal Th1 exhibited upregulation of Lt-b, TNFR1, and TNFRSF11A, and Th2 showed an additional upregulation of myosin light chain (MLC)-1, an antiapoptotic factor. It should be noted that anti-IL-4 antibody inhibited the upregulation of TNFR1 and TNFRSF11A *(952)*. Therefore, the increased apoptosis observed during the secondary response of Th1 may be related to a defective expression of MLC-1.

Studies of in vitro maturation of cord blood lymphocytes also suggest a biased response of human neonatal CD4 T cells toward Th2 cells. Naïve neonatal T lymphocytes contain a subset characterized by $CD4^+$ $CD31^-$ $CD45RO^-$ cells, which differentiate into IL-4-producing cells after long-term culture in IL-4 and IL-12 supplemented medium or stimulation with low doses of anti-CD28 antibodies *(953)*.

The biased development toward Th2 cells in newborns may be highly influenced by immune status during pregnancy. In mice, fetoplacental tissue is characterized by secretion of Th2 cytokines such as IL-4, IL-5, IL-10, PGE2, and progesterone, which downregulate the production of IL-12, promoting the development of Th1 cells (data reviewed in ref. *953*).

Overall, these studies indicate that neonatal T cells can respond to antigen stimulation in vitro and in vivo and that priming with antigen stimulates both

Th1 and Th2 responses. However, the secondary response of neonates is biased to Th2 responses. The biased neonatal Th2 response may be related to IL-2-induced apoptosis of Th1 cells in a recall response.