CAAGCTTGGAAATTGATGAG-3′ and 5′-CTGG-TACCAACTGAAAGAAG-3′; $J_\delta1$, 5′-GGAATTCCT-TCTGCAAATACCTTG-3′ and 5′-AGAGGGAAT-TCCTATGAGCT-3′; $V_\delta1$, 5′-AATAGGAATTCTAC-TGATGGTGG-3′ and 5′-ATGGATCCAATGCTCT-GTTTTTAG-3′; $J_\delta1$, 5′GTGGGATCCTTGTCCAAA-GAC-3′ and 5′-GTGGATCCACAGTCACTTG-3′; $J_\delta2$, 5′-GCCGGATCCAAAAAACATCTG-3′ and 5′-GGGATCCACAAAGAGCTC-3′; V_HS107, 5′-CTG-GAATTCGAAACAAAGCTAATG-3′ and 5′-TCTG-GAGGAGGCTTGGTACA-3′; V_H81X, 5′-CCTGT-GAATCCAATGAATACG-3′ and 5′-GTGGAGTCTG-GGGGAGGCTTA-3′; and J_H3, 5′-TGCAGAGAATC-TTGGTCCTG-3′ and 5′-ACTTCAAGCTTCAGTTC-TGG-3′. Twenty-five to 30 cycles of amplification were done with a programmable thermal controller (MJ Research, Inc.) (1 min at 94°C, 1 min at 52°C to 60°C, and 30 s to 1 min at 72°C). A second round of amplification was done starting with 2 to 15 μl of the first-round mixture [M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White. Eds., *PCR Protocols: A Guide to Methods and Applications* (Academic Press, San Diego, CA, 1989), pp. 21–27].
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29. To construct the targeting vector, we cloned a 0.7-kb genomic Eco RI–Hind III fragment 5′ of the TdT gene into pBluescript SK (Stratagene) containing a 2.7-kb Eco RI–Hind III PGK–HSV-*tk* fragment in the Sst I site. A 1.6-kb Eco RI–Bgl II PGK-*neo* fragment was blunt end–ligated in the opposite transcriptional orientation into the Hind III site. To obtain the final targeting vector, we inserted an 8-kb genomic Xba I*–Eco RI and 7-kb genomic Xho I–Kpn I* fragment respectively, into the Eco RI site and Xho I site of this construct (asterisk denotes sites in cloning vector). ES cells (CCE, 2 × 10^7) were transfected with Kpn I–linearized targeting vector (20 μg) and selected with G418 (0.4 mg/ml) and gancyclovir (1 μM) (*16*). Three hundred and sixty-two selected clones were screened by Southern (DNA) blot analysis of Eco R I–digested genomic DNA probed with the Sal I–Xba I fragment 3′ of the region included in the targeting construct to identify 43 $TdT^{+/-}$ clones. Four $TdT^{+/-}$ clones were selected in increased G418 concentration (1.6 to 4 mg/ml) to obtain $TdT^{-/-}$ clones (*17*). Subclones of single ($TdT^{+/-}$) or double ($TdT^{-/-}$) mutated ES cells were injected into blastocysts of RAG-2–deficient blastocysts and transferred into $B6CBAF_1/J$ (Jackson Laboratory) females (*18*).
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Mice Lacking TdT: Mature Animals with an Immature Lymphocyte Repertoire

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In adult animals, template-independent (or N) nucleotides are frequently added during the rearrangement of variable (V), diversity (D), and joining (J) segments of lymphocyte receptor genes, greatly enhancing junctional diversity. Receptor genes from adult mice carrying a mutation in the terminal deoxynucleotidyl transferase (TdT) gene have few N nucleotides, providing proof that this enzyme is essential for creating diversity. Unlike those from normal adults, receptor genes from adult mutant mice show extensive evidence of homology-directed recombination, suggesting that TdT blocks this process. Thus, switch-on of the TdT gene during the first week after birth provokes an even greater expansion of lymphocyte receptor diversity than had previously been thought.

The repertoire of B and T cell antigen receptors expressed in adult animals is more diverse than that in perinates (*1*). One major difference is the amount of N region diversity at the junctions of rearranged immunoglobulin (Ig) and T cell receptor (TCR) gene segments. N nucleotides are rare in V(D)J junctions from fetal or newborn animals, but constitute a major component of the diversity of Igs and TCRs from adults (*2–7*). This dissimilarity may be due to differential expression of TdT. Terminal deoxynucleotidyl transferase catalyzes template-independent addition of nucleotides in vitro (*8*), and the amount expressed in vivo correlates with the degree of N region diversity in antigen receptors (*9*, *10*). Another difference between adult and perinatal repertoires lies in the diversity of V-J, V-D, and D-J junctional sequences. Examination of large sets of fetal and newborn Ig and γδ TCR sequences revealed overrepresentation of some junctions, coincident with short stretches of homology between abutted gene segments (*3–5*, *11–13*). In the case of γδ TCRs, certain dominant junctions (termed "canonical") are functionally significant because they give rise to the quasi-monoclonal receptors in specific anatomical locations such as the skin. Overrepresented joints were not generally observed in adult sequences, only in some of those lacking N nucleotides. Initially, the presence of dominant junctions of γδ TCRs was attributed to cellular selection (*5*, *14*), but a preference for rearranging at short stretches of homology is more probable (*15*, *16*). Why such homology-directed recombination is pronounced in perinates but rare in adults is an open question.

One approach to better understanding the adult-perinate dichotomy is to artificially produce mature animals with repertoires having immature features. Thus, we generated, through homologous recombination in embryonic stem cells, a strain of mice lacking TdT (*17*). The mutation of TdT we obtained was an insertion of the neomycin gene into exon 4, as illustrated in Fig. 1 and confirmed by extensive Southern (DNA) blot analysis. Given the predicted location of the TdT active site and its presumed globular nature (*18*), exons 4 to 7 are probably critical for TdT function. No mRNA corresponding to regions 3′ of the neomycin insert was detected in thymus RNA from homozygous mutant mice after polymerase chain reaction (PCR) amplification, for which we used a primer pair on the 3′ side of the insertion; nor was any revealed by in situ hybridization of the appropriate probe to thymic sections (*19*). Abrogation of protein expression was confirmed by staining of thymocytes with a polyclonal antiserum to TdT (*19*).

Homozygous mutant $TdT^{-/-}$ mice breed well and appear healthy in a conventional animal facility, are of normal size, and do not have increased susceptibility to infection, as is common for immunodeficient animals in our colony. The mutants show no marked abnormalities in the major T or B cell compartments and are capable of mounting T and B cell responses to complex antigens like keyhole limpet hemocyanin and ovalbumin (*20*).

To evaluate the effect of a TdT deficiency on the lymphocyte repertoires of adult mice, we sequenced the V(D)J junctions of more than 300 rearranged Ig and TCR genes from adult animals (most from 6 to 8 weeks of age) (*21*). Representative sets of $V_\gamma3$ DNA sequences from total thymocytes (Fig. 2), V_H7183 DNA sequences from splenocytes (Fig. 3), and $V_\beta8$ RNA sequences from $CD4^+$-$CD8^+CD3^{lo}$ thymocytes (Fig. 3) are shown. The enzyme TdT was responsible for the bulk of N region diversity because

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the abundant N nucleotides found in rearranged genes of wild-type mice were absent in the mutant. Nonetheless, a few nucleotides not encoded in the germline do occur in these and our other data sets from $TdT^{-/-}$ mice; of the junctions that can be unequivocally assigned (the relevant V_γ, J_γ, D_H, J_H, V_β, D_β, and J_β genomic sequences being on record), about 3% had inserts of one to three nucleotides. Although PCR and sequencing errors may account for a few of these extra nucleotides, it is unlikely that such errors would be clustered so precisely at the joints. Similar inserts have been noted after rearrangement events involving other genes in nonlymphoid cell types. For example, about 10% of the junctions arising by circularization of transfected linear DNA have extra nucleotides (*22*). At least two mechanisms can be invoked to explain insertions in the absence of TdT: (i) incorporation of oligonucleotides during the joining process and (ii) nucleotide misincorporation by DNA polymerase at free ends during the fill-in and repair process that presumably occurs before joining. Some prokaryotic and eukaryotic DNA polymerases add one or two extra nucleotides when copying an oligonucleotide substrate (*23*). Despite the few template-independent nucleotides observed, we can now say with some certainty that TdT is the enzyme responsible for the difference in N region diversity characteristic of perinatal and adult antigen receptor repertoires, confirming earlier predictions (*9*, *10*).

Analysis of the mutant mice also permitted us to evaluate the role of TdT in other aspects of the repertoire, in particular homology-directed recombination, as defined above. Rearranged $V_\gamma3$-$J_\gamma1$ genes seem best suited for studying this aspect because three V-J junctions are significantly overrepresented in the fetal thymus. One junction, which comprises about 40% of the sequences, is in-frame and results in the invariant receptor found on essentially all intraepithelial lymphocytes in the skin [termed junction 1 by Itohora *et al.* (*15*)]; the two others, each comprising about 20% of the sequences, are out-of-frame (designated junctions 2 and 3). The dominance of these "canonical" junctions reflects a preference for recombining at short stretches of nucleotide homology (*15*, *16*). Overrepresented joints are not a feature of the repertoire generated in adults. To determine whether TdT influences homology-directed recombination, we analyzed $V_\gamma3$-$J_\gamma1$ sequences derived from pairs of $TdT^{-/-}$ and TdT^+ littermates. The sequences were obtained from either total thymocyte DNA or DNA from sorted populations of $CD4^-CD8^-$ and $CD4^+CD8^+$ thymocytes, enriched for cells committed to the γδ or αβ lineage, respectively. In all of the data sets from $TdT^{-/-}$ animals (for example, Fig. 2), canonical joints were overused: junction 1 in 24 to 30% of the sequences, junction 2 in 20 to 23%, and junction 3 in 11 to 13% (Table 1). The data from sorted thymocyte populations indicate that this is true of cells from the αβ as well as the γδ lineage. In contrast, these joints are rare in the rearranged $V_\gamma3$-$J_\gamma1$ genes from TdT^+ animals. According to current models of the rearrangement process (*24*), their use would result in genes lacking N region diversity because homology alignment would be the last step before ligation. The large major-

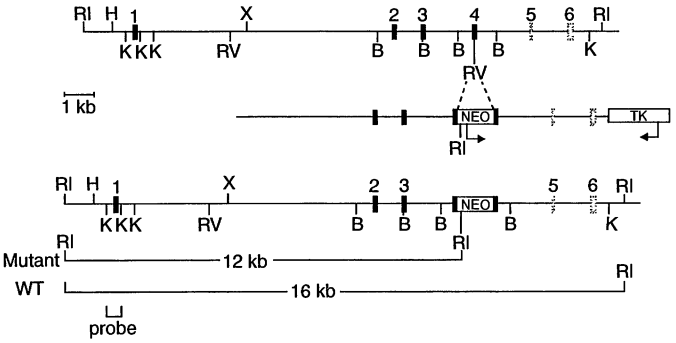

Fig. 1. Disruption of the TdT gene. A partial restriction map of the TdT gene, targeting vector, and mutant allele are shown. The positions of exons 5 and 6 are approximate (within 1.5 kb). For construction of the targeting vector, a 1.1-kb fragment of the neomycin resistance gene (pMC1neo-polyA, Stratagene) was cloned into the Eco RV site in exon 4 of the 11-kb Xho I–Kpn I TdT genomic fragment, and the herpes simplex virus thymidine kinase gene was appended to the 3′ end to allow double selection. RI, Eco RI; RV, Eco RV; K, Kpn I; X, Xho I; B, Bgl II; and H, Hind III.

TCR γ

TdT^+ $V_\gamma3$	P	N	P	$J_\gamma1$		TdT^- $V_\gamma3$	P	N	P	$J_\gamma1$	
in frame						in frame					
TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1	TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1	TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
TGTGCCTGCTGG			T	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
TGTGCCTGCTGG		TTA		AGCTCGGGTTTT		TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
TGTGCCTGCTGGGATC		CCTC	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
out of frame						TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
TGTGCCTGCTGGGAT			AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				AGCTCAGGTTTT	#1
TGTGCCTGCTGGGAT			AT	ATAGCTCAGGTTTT		TGTGCCTGCT				CAGGTTTT	
TGTGCCTGCTGGGATC				TAGCTCAGGTTTT	#2	TGTGCCTGCTGGGATC				GCTCAGGTTTT	
TGTGCCTGCTGG			AT	ATAGCTCAGGTTTT		out of frame					
TGTGCCTGCTGGGA			AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGGTC				TAGCTCAGGTTTT	#2
TGTGCCTGCTGG				ATAGCTCAGGTTTT		TGTGCCTGCTGGGATC				TAGCTCAGGTTTT	#2
TGTGCCTGCTGGGA		CCCCATCA	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGATC				TAGCTCAGGTTTT	#2
TGTGCCTGCTGGGATCT		GGG		AGCTCAGGTTTT		TGTGCCTGCTGGGATC				TAGCTCAGGTTTT	#2
TGTGCCTGCTGGGATC		CGT	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGATC				TAGCTCAGGTTTT	#2
TGTGCCTGCTGGGAT		T		TAGCTCAGGTTTT		TGTGCCTGCTGGGATC				TAGCTCAGGTTTT	#2
TGTGCCTGCTGGG		GGG		AGCTCAGGTTTT		TGTGCCTGCTGGGAT				ATAGCTCAGGTTTT	#3
TGTGCCTGCTGGG		G	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				ATAGCTCAGGTTTT	#3
TGTGCCTG		G	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				ATAGCTCAGGTTTT	#3
TGTGCCTGC		TC	T	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				ATAGCTCAGGTTTT	#3
TGTGCCTG		GG	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGAT				ATAGCTCAGGTTTT	#3
TGTGCCTGCTGGGATC		CGA	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGG			AT	ATAGCTCAGGTTTT	
TGTGCCTGCTGGGATCT		GGGGG		GGTTTT		TGTGCCTGCTGG			AT	ATAGCTCAGGTTTT	
TGTGCCTGCTGGG		TC		ATAGCTCAGGTTTT		TGTGCCTGCT				AGCTCAGGTTTT	
TGTGCCTGCTGGGAT		GAGGGG		TAGCTCAGGTTTT		TGTGCCTGCT				AGCTCAGGTTTT	
TGTGCCTGCTG		AAGGC		AGCTCAGGTTTT		TGTGCCTGCTGG				ATAGCTCAGGTTTT	
TGTGCCTGCTGGG		GGAA		ATAGCTCAGGTTTT		TGTGCCTGC			AT	ATAGCTCAGGTTTT	
TGTGCCTGCTGGG		GC		ATAGCTCAGGTTTT		TGTGCCTGCTGG				CTCAGGTTTT	
TGTGCCTGCTGGGA		C	AT	ATAGCTCAGGTTTT		TGTGCCTGCTGGGATC			AT	ATAGCTCAGGTTTT	
TGTGCCTGCTGGGA		AAN		ATAGCTCAGGTTTT		TGTGCCTGCT				TAGCTCAGGTTTT	
TGTGCCTGCTGGGATCT		TTGG		AGCTCAGGTTTT		TGTGCCTGCT			AT	ATAGCTCAGGTTTT	
TGTGCCTGCTG		AAGGC		AGCTCAGGTTTT		TGTGCCTGCTGG				CTCAGGTTTT	
TGTGCCTGCTGGG		GT	AT	ATAGCTCAGGTTTT		TGTGCCT			AT	ATAGCTCAGGTTTT	
TGTGCCTGCTGGG		GTC	T	ATAGCTCAGGTTTT		TGTGCCTGCTGG		T		CTCAGGTTTT	
TGTGCCTGCTGGG		CT		ATAGCTCAGGTTTT							

Fig. 2. Representative sequences of $V_\gamma3$-$J_\gamma1$ junctions from age-matched adult mice, either wild-type TdT^+ (o/+ or +/+ genotypes) or mutant $TdT^{-/-}$ (o/o genotype). The TCRγ sequences were derived from total thymus DNA. The assignment of N nucleotides was based on the known sequences of the germline elements. Homologies of two or more nucleotides shared between joined segments are underlined. We did not score putative homologies of a single base because their significance is difficult to ascertain: in the absence of homology-induced joining, the probability of any junction containing a single-nucleotide "homology" is 38%. Homology was assigned assuming possible P inserts of up to five bases. P inserts are template-dependent nucleotides added to the ends of recombining gene segments; they are palindromic to the terminal few nucleotides of the unadulterated coding joint (*5*). Repeated junctions are shown only if they were obtained in independent amplifications from at least two mice. For $V_\gamma3$, the canonical junctions are numbered according to (*15*).

ity of sequences from TdT$^+$ animals carry N nucleotides, and less than half of those that lack N additions have a canonical junction. It might also be worth mentioning, should the currently preferred model of rearrangement prove incorrect, that the sequences with N nucleotides almost never have the same cutting points as those used to generate the canonical junctions.

Extensive use of homology-directed recombination has also been documented for the Ig heavy chain genes of perinatal mice (*3, 4, 11, 12*). Therefore, we analyzed V_H7183-J_H4 and V_HJ558-J_H2,3 sequences to see whether our observations on rearranged V_γ3 genes could be generalized (Fig. 3). The representative data set shows the frequent use of a few junctions—particularly certain D-J joints—in the sequences from TdT$^{-/-}$ mice, all of which are situated at short stretches of nucleotide homology. Overused junctions are rare in sequences derived from TdT$^+$ animals.

Analysis of rearranged TCRβ genes revealed a different situation (Fig. 3 and Table 1). Homology-directed recombination is not a dominant feature of the V_β repertoire in either TdT$^{-/-}$ or TdT$^+$ mice. In both types of mice, junctions are heterogenous: almost every sequence has a different V-D and D-J joint. That such heterogeneity is a feature common to V_β sequences, whether or not they show N region diversity, was confirmed by examination of published perinatal and adult V_β5,6,8 and 17a genes (*6, 7, 10*).

Two conclusions can be drawn from the data on homology-directed recombination. First, the presence of TdT somehow blocks this process. This could be a direct effect at the protein level—for example, the binding of TdT to the ends of segments might inhibit attachment of some protein needed to hold together short homologous stretches of nucleotides. Our data and those of others (*4, 12*) are consistent with this possibility: adult sequences devoid of N region diversity consistently have fewer dominant junctions than do perinatal sequences lacking N nucleotides. The blocking of homology-directed recombination of TdT could also be an indirect effect; the addition of nucleotides to segment ends might set stretches of homology too far apart for the recombination machinery to join them effectively. This possibility has been suggested previously (*12*) and is supported by the preponderant use of homologies residing close to the ends in both Ig and TCR γδ genes. A similar preference for homologous stretches near the extremities was observed after circularization of transfected linear plasmids in nonlymphoid cells (*25*).

The second point is that in animals whose receptor variety is severely restricted by the absence of N region diversity, some receptor gene families (V_γ3-J_γ1 and IgH) use a strategy to further reduce variety, whereas the TCR genes do not. The D_β and J_β segments actually seem to be organized to avoid homology-directed recombination. Few stretches, even dinucle-

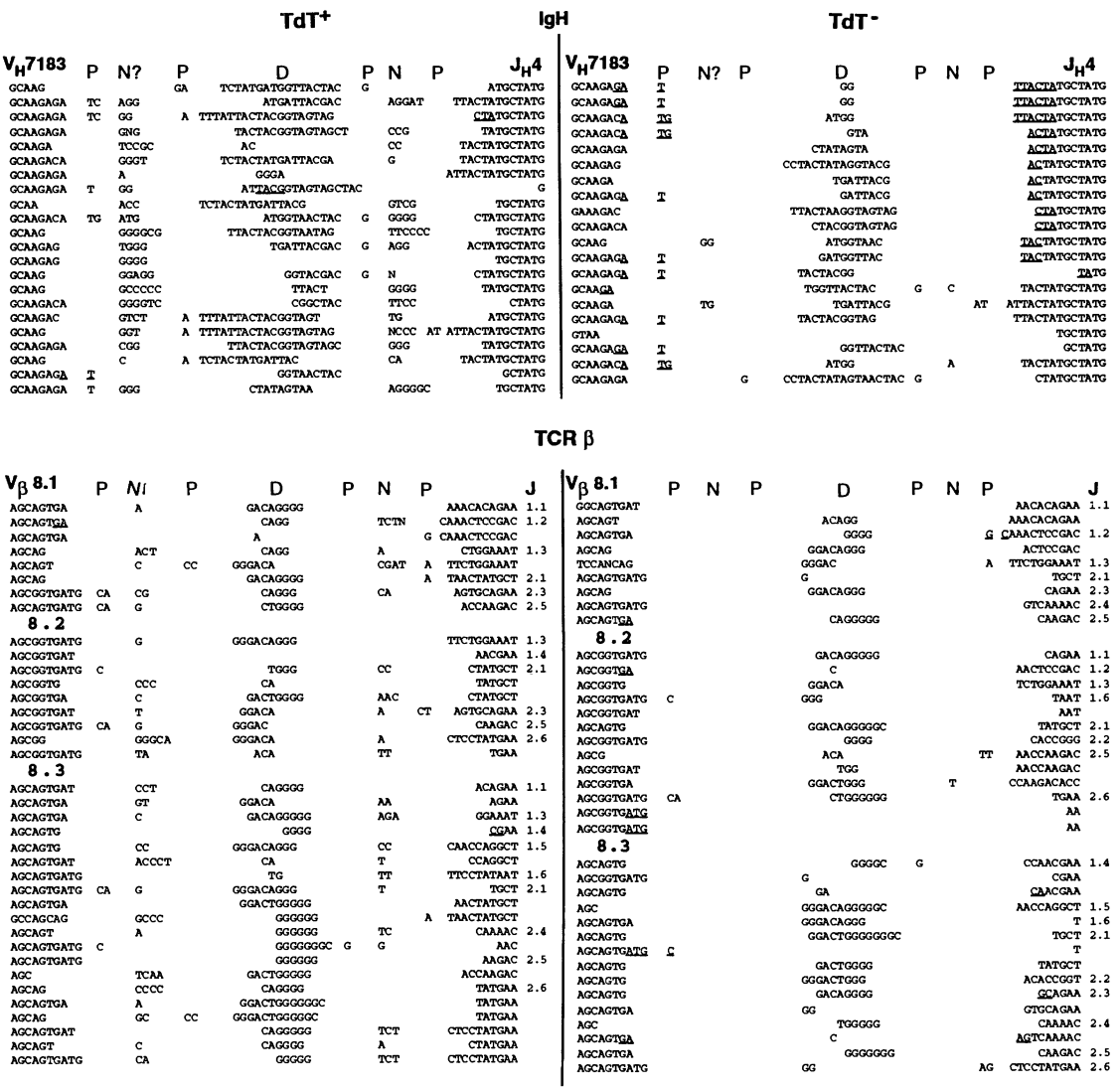

Fig. 3. Representative sequences of V_H7183-D-J_H4 and V_β8-D-J_β junctions from age-matched mice, either wild-type (TdT$^+$) or mutant (TdT$^{-/-}$) as in Fig. 2. Immunoglobulin heavy chain sequences came from total spleen DNA and TCRβ sequences from CD4$^+$CD8$^+$CD3^{lo} thymocyte RNA. Assignment of N nucleotides was as in Fig. 2, except for the several members of the V_H7183 family for which the germline sequences are not known; in this instance, we relied on the fact that those V_H7183 family members whose germline sequences are known end with either . . .GCAAGACA or . . .GCAAGAGA (*12*). Underlined bases are homologies of two or more nucleotides. Homologs were scored as in Fig. 2. The impression that the D segment is generally shorter in TdT$^{-/-}$ mice is an illusion caused by the routine placement of homologous nucleotides in the V or J segments.

Table 1. Use of homology in TdT$^{-/-}$ and TdT^{+} V(D)J junctions. Most data sets were compiled from independent amplifications from at least two mice. Only homologies of two nucleotides or more were counted, and as in the figures we considered the possibility of P inserts of up to five nucleotides. The $V_\gamma 3$ canonical junctions are numbered as in (*15*). DP, CD4^{+}CD8^{+}; DN, CD4^{-}CD8^{-} thymocytes.

	$V_\gamma 3$			$V_\beta 8$ DP CD3 lo	V_H 783/J558 spleen (D-J only)
	Total	DP	DN		
		TdT^{-}			
No. of junctions	64	54	46	122	39
No. of junctions with N	3	0	3	3	2
No. of homologies > 1 bp (% in N^{-} junctions)	40 (66)	35 (65)	29 (67)	22 (18)	20 (54)
No. of canonical junctions (% total junctions)					
Junction 1	17 (27)	16 (30)	11 (24)		
Junction 2	15 (23)	11 (20)	9 (20)		
Junction 3	7 (11)	7 (13)	5 (11)		
Total	39 (61)	34 (63)	25 (54)		
		TdT^{+}			
No. of junctions	55	54	30	82	23
No. of junctions with N	42	38	12	50	17
No. of homologies > 1 bp (% in N^{-} junctions)	6 (46)	7 (44)	8 (44)	2 (6)	2 (33)
No. of canonical junctions (% total junctions)					
Junction 1	2 (4)	1 (2)	0		
Junction 2	2 (4)	1 (2)	5 (17)		
Junction 3	2 (4)	5 (9)	3 (10)		
Total	6 (11)	7 (13)	8 (27)		

otides, are shared between the two: the D segments are essentially G nucleotide stretches, whereas few G nucleotides are found in the J segments. Consequently, TCRβ genes will be very diverse even in perinatal animals.

Thus, the enzyme TdT catalyzes the bulk of N nucleotide addition to V, D, and J segment ends and blocks homology-directed recombination. These two processes greatly magnify the number of antigen receptor specificities expressed by adults as compared with perinates. It remains to be determined which is more critical for the development and well-being of the animal, the relatively restricted perinatal or the highly diverse adult repertoire.

The accompanying paper by Komori *et al.* arrived at similar conclusions about mice carrying a different TdT mutation (*26*).

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17. To produce TdT$^{-/-}$ mice, we constructed a targeting vector as illustrated in Fig. 1. It was linearized and electroporated into D3 embryonic stem cells as described (*27*). Of 151 Gancyclovir/G418-resistant D3 clones analyzed, one carrying the predicted integration was identified, and this was confirmed by extensive Southern blot analysis. It was expanded and injected into blastocyts, which were reimplanted into pseudopregnant females. Chimeras were crossed to C57Bl/6 mice, and offspring carrying the mutation were intercrossed to produce homozygous TdT$^{-/-}$ mice. The presence of the mutation was confirmed by Southern blot analysis of Eco RI–, Eco RV–, or Bgl II–digested tail DNA with the use of a PCR-generated cDNA probe spanning exons 4 to 6. Mice were housed in a conventional animal facility and handled according to EEC guidelines.
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20. The immune system of TdT$^{-/-}$ mice was characterized (S. Gilfillan *et al.*, in preparation).
21. DNA and RNA were isolated from sorted cells and tissues with the use of standard techniques. Briefly, thymocyte suspensions from individual mice were stained with appropriate combinations of monoclonal antibodies to CD4, CD8 (Caltag), and CD3 (KT3) (*28*) and electronically sorted (*29*). RNA was prepared by NP-40 lysis from 1×10^5 to 2×10^5 sorted cells to which 1×10^6 HeLa cells were added as carrier; DNA was isolated directly from 1×10^5 to 5×10^6 sorted cells. RNA was isolated from spleen tissue and cDNA synthesized as described (*29*). DNA was prepared by proteinase K digestion followed by phenol-chloroform extraction. PCR was carried out in a DNA Thermal Cyler (Perkin-Elmer/Cetus); products were digested with appropriate restriction enzymes, cloned into M13mp19, and sequenced by either the dideoxy method or by automated sequencing (Applied Biosystems). The conditions and primers (restriction sites are underlined) used were as follows: $V_\beta 8$, QN199 (GAGGAAAGGTGACATTGAGC) (*7*) and QN200 (CGACGCATGCTGGTATCGGCAGGAC) (*7*); C_β, MQ284 (AGCACACGAGGGTAGCCTT) (*29*) and MS175 (GACAGAACTTTGAATTCCTCTGCTTTTGATGG) (*29*); $V_\gamma 3$, QR239 (CACCATTCTGCAGTGGTACCAACTG) (*5*) and OU47 (GTCCTGACCTCCTGCAGCCGCTTGGAAATT) (*30*); $J_\gamma 1$, QR238 (CAGAGGGAATTCCTATGAGCTTAGT) (*5*); $V_H 7183$, QR194 (CGCGCTGCAGCGTGGAGTCTGGGGGAGGCTTA) (*31*) and QU234 (GTGCAGCCTGCAGAGTCCCTGAAACTCTCC); $J_H 4$, QR240 (CCTGGAGAGGGAATTCTTACCTGAGGAG) and QQ4 (GCGCTCGAGAATTCGGTGACTGAGGTT) (*31*); $V_H J558$, QQ3 (GTCCAACTGCAGCAGCCTGGGGCTGAG); and C_μ, QQ2 (GGGGAATTCATTTGGGAAGGACTGACT) (*4*). For $V_\beta 8$, cDNA was amplified as described (*7*). We amplified $V_\gamma 3$ and $V_H 7183$ junctions from 1 to 2 μg of Eco RI–digested DNA using two rounds of PCR. For the primary amplification, samples were denatured for 6 min at 94°C and then subjected to 25 amplification cycles—each consisting of 30 s at 94°C, 1 min at 55°C, and 1 min at 72°C—followed by a 10-min elongation at 72°C. One microliter of the primary reaction was used for the secondary PCR in which the annealing step was changed to 30 s at 60°C. We amplified $V_H J558$ junctions from cDNA using a single set of V_H and C_μ primers as follows: 6 min denaturation at 94°C; 25 cycles of 45 s at 94°C, 1 min at 60°C, and 2 min at 72°C; and a final elongation of 10 min at 72°C.
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