

Most α/β T Cell Receptor Diversity Is Due to Terminal Deoxynucleotidyl Transferase

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Abstract

The contribution of template-independent nucleotide addition to antigen receptor diversity is unknown. We therefore determined the size of the T cell receptor (TCR) α/β repertoire in mice bearing a null mutation on both alleles of the terminal deoxynucleotidyl transferase (Tdt) gene. We used a method based upon polymerase chain reaction amplification and exhaustive sequencing of various AV-AJ and BV-BJ combinations. In both wild-type and Tdt^{0/0} mice, TCRAV diversity is one order of magnitude lower than the TCRBV diversity. In Tdt^{0/0} animals, TCRBV chain diversity is reduced 10-fold compared with wild-type mice. In addition, in Tdt^{0/0} mice, one BV chain can associate with three to four AV chains as in wild-type mice. The α/β repertoire size in Tdt^{0/0} mice is estimated to be 10⁵ distinct receptors, ~5–10% of that calculated for wild-type mice. Thus, while Tdt activity is not involved in the combinatorial diversity resulting from α/β pairing, it contributes to at least 90% of TCR α/β diversity.

Key words: T cell repertoire • T cell receptor • knockout mice • terminal deoxynucleotidyl transferase • CDR3

Introduction

T cell function relies on the specific recognition of foreign antigens. It is assumed that the larger the number of distinct immune T cells, the more efficient the protection against infectious diseases. The diversity of antigen receptors is generated through combinatorial and junctional mechanisms (1). The combinatorial diversity of variable regions is obtained by random recombination between V, D, and J gene segments. Pairing between heavy and light chain for Ig, α and β , or γ and δ chains for TCR adds another level of diversity. Deletion or addition of nucleotides at the coding ends of V(D)J gene segments during the recombination process results in junctional diversity. Template-dependent nucleotide addition (P regions) comes from the asymmetrical opening of the hairpin structure (2) whereas template-independent nucleotide addition (N regions) is mediated by the enzyme terminal deoxynucleotidyl transferase (Tdt) (3–5). Tdt adds nucleotides at 3' ends of each coding gene segment (6). Each TCR junction bears 2 to 3 nucleotides on the average (for a review, see reference 7). In the thymus, Tdt expression is developmentally regulated (8). Tdt

mRNA is detected in the thymus by day 4 after birth and N-regions appear 1 or 2 d later in immature thymocytes undergoing differentiation (9).

Tdt-driven junctional diversity is crucial for the TCR α/β repertoire because the limited number of available gene segments when compared with those of the Ig repertoire significantly reduces the combinatorial diversity. Furthermore, both TCR AV and BV chains have N regions whereas only Ig heavy chains contain N additions. Interestingly, mice with a null mutation of the Tdt gene (Tdt^{0/0}) (4) are not immunodeficient (10). Using a large panel of immunological assays, no significant differences were found between Tdt-deficient and wild-type (wt) mice. Moreover, Tdt^{0/0} animals infected by the mouse pathogen lymphocytic choriomeningitis virus (LCMV) or contaminated in a conventional colony by Sendai virus recovered from these infections (10). Gavin and Bevan (11) demonstrated that TCR lacking N additions are more promiscuous than TCR with N additions. Taken altogether, these functional results raised the question of the actual repertoire size available in these Tdt^{0/0} mice: to what extent is the repertoire size reduced and are there compensatory mechanisms at work allowing the maintenance of a large enough diversity to sustain normal immune responses?

Our group has recently developed a method to estimate the size of the TCR α/β repertoire in human blood T lym-

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phocytes (12) and in mouse T splenocytes (13). We have used this methodology, i.e., PCR amplification and extensive sequencing, to determine the size of the TCR α/β repertoire in Tdt $^{\circ}/^{\circ}$. The results presented herein provide the first quantitative estimate of the impact of Tdt on AV and BV diversity.

Materials and Methods

Animals. All mice used in this study were 6-wk-old C57Bl/6 Tdt $^{\circ}/^{\circ}$ (4) or C57Bl/6 mice raised in specific pathogen-free (SPF) conditions and obtained from the Pasteur Institute housing facilities and IFFA-Credo (l'Arbresle, France), respectively.

Antibodies and Sorting of T Splenocytes. FITC-labeled anti-V α 2, anti-V α 8, and anti-CD44, PE-labeled anti-TCR β , anti-V β 7, and anti-V β 10, and tricolor-labeled anti-CD62L antibodies were purchased from BD Pharmingen; biotinylated anti-B220 was from Caltag.

Splenocytes from 6-wk-old Tdt $^{\circ}/^{\circ}$ or C57Bl/6 males were depleted of B220 $^{+}$ cells. B220-negative splenocytes were incubated with indicated antibodies at 4°C. Cells were sorted on an Epics-Elite ESP (Coultronics) at the Flow Cytometry Unit (Institut Jacques Monod, Paris, France). Cell purity after sorting was analyzed by flow cytometry and was above 98% in both samples.

RNA Extraction and cDNA Synthesis. Unfractionated or sorted T splenocytes from Tdt $^{\circ}/^{\circ}$ and C57Bl/6 mice were used for RNA preparation. Total RNA from splenocytes was extracted and reverse-transcribed into cDNA using random primers (5 μ M) or oligo(dT) (13).

Immunoscope Analysis. PCR were performed in 50 μ l on 1/50 of the cDNA with 2 U of Taq polymerase (Goldstar) in the supplier's buffer. cDNA was amplified using BV/BC or AV/AC specific primers. Amplified products were used as template for an elongation reaction with fluorescent tagged oligonucleotides (14).

Cloning and Sequencing of TCRBV and TCRAV Rearrangements. The cloning and sequencing method has been described (13). Briefly, PCR were performed with 5 U Pfu polymerase (Stratagene) in the supplier's buffer. PCR products were visualized by a DNA silver staining system (Promega). DNA from bands corresponding to a given CDR3 length was extracted and subjected to a second PCR. PCR products were then cloned in pCR $^{\circ}$ 4Blunt TOPO vector using the Zero blunt TOPO PCR cloning kit (Invitrogen). Alternatively, PCR products were cloned in pCR $^{\circ}$ 4Blunt TOPO vector without separation of CDR3 bands on acrylamide gels.

For sequencing purposes, PCR was performed directly on LacZ $^{-}$ colonies with Taq polymerase (13). Sequencing reactions were performed on these products using M13 (-20) primer with the ABI PRISM Big Dye Terminator Reaction Kit (Applied Biosystems). CDR3 region corresponding sequences were extracted and analyzed using software designed for this purpose (13).

Quantitative PCR Assay. Quantitative PCR were performed in the GeneAmp $^{\circ}$ 5700 (Applied Biosystems) (15) in 25 μ l on 1/20 of the cDNA preparation with 1 U of Taq polymerase in the supplier's buffer as performed by Lim (personal communication). cDNA was amplified using specific sense primer of the AV2 segment (CAATAAAAGGGAGAAAAAGCTCTCC) and antisense primers hybridizing in AC or AJ44 segments, respectively (ACACAGCAGGTTCTGGGTTTC and GTCCAAAC-GTGAGCTTTCCAGT). We also used a fluorogenic oligonucleotide (TCCAGGCTGAGAGTCTGTGATGTGCA) specific for the AV2 segment in which a reporter fluorescent dye on 5'

(FAM $^{\text{TM}}$) and a quencher dye on 3' (TAMRA $^{\text{TM}}$) were attached. The specific activities of AJ44 and AC primers were tested using a cDNA from a T cell hybridoma bearing an AV2-AJ44 rearrangement. They were compared by the comparative C $_T$ method as described by the manufacturer. We have calculated the 2 $^{-\Delta C_T}$ and found values close to 1 indicating that the specific activities of these primers can be considered identical.

Statistical Calculations. The equation used by Barth et al. (16) and Behlke et al. (17) enabled us to estimate the maximum probable number of distinct CDR3 sequences found in the cDNA preparation. Namely, the maximum likelihood estimate (MLE) of the number of distinct sequences was calculated as described previously (13).

Calculation of Repertoire Size. The different methods of repertoire calculation have been described (13). Size of the BV repertoire = number of distinct sequences found in all CDR3 peaks (MLE value) divided by (frequency of BV \times frequency of BJ segment). Size of the TCR α/β repertoire = number of distinct sequences found in all CDR3 peaks (MLE value) from the AV2 $^{+}$ T cells divided by (frequency of BV \times frequency of BJ segment \times frequency of AV).

Results and Discussion

Immunoscope analyses of the T cell repertoire allow the visualization of the distribution of TCR CDR3 lengths for each AV-AC and BV-BC rearrangement, as a series of six to eight bands resolved on acrylamide gel. In nonimmunized mice, the CDR3 length distributions, are mainly centered around a CDR3 length of 9 or 10 AA, and adopt a Gaussian-like profile which is the hallmark of a polyclonal, naive T cell repertoire.

In Fig. 1 are presented the CDR3 length profiles obtained for 6 BV-BC and 3 AV-AC combinations, using cDNA from T splenocytes of nonimmunized, SPF C57Bl/6 or C57Bl/6 Tdt $^{\circ}/^{\circ}$ mice. All combinations tested (10 AV-AC and all functional BV-BC) exhibit Gaussian-like curves in C57Bl/6 or Tdt $^{\circ}/^{\circ}$ animals, indicating that the T cell repertoire in Tdt $^{\circ}/^{\circ}$ mice is polyclonal. In addition, profiles using fluorescent BJ primers again show Gaussian-like curves for each BV tested (data not shown). Altogether, these data show that the absence of Tdt did not bias the T cell repertoire toward the preferential usage of some AV or BV gene segments.

One striking feature of the Tdt $^{\circ}/^{\circ}$ BV-BC and AV-AC profiles is a shift toward shorter CDR3 lengths. The majority of BV-BC profiles shows a 1 amino acid (AA) reduction of their CDR3 length while the CDR3 of some BVs (i.e., BV1, BV6, BV7, BV8.1, BV8.3, BV14) are shorter by 2 AA compared with wt mice (Fig. 1 B and data not shown). On the other hand, 10 AV-AC combinations (Fig. 1 A, and data not shown) in Tdt $^{\circ}/^{\circ}$ mice show a shift toward shorter CDR3 lengths by at most 1 AA only. This difference between AV and BV chains is consistent with the average number of added nucleotides at each junction (7). This suggests that the CDR3 β lengths are longer than the CDR3 α ones owing to the presence of 4 sites available for Tdt during BV chain rearrangement instead of 2 for AV chains. The reduction of the CDR3 length in Tdt $^{\circ}/^{\circ}$ mice

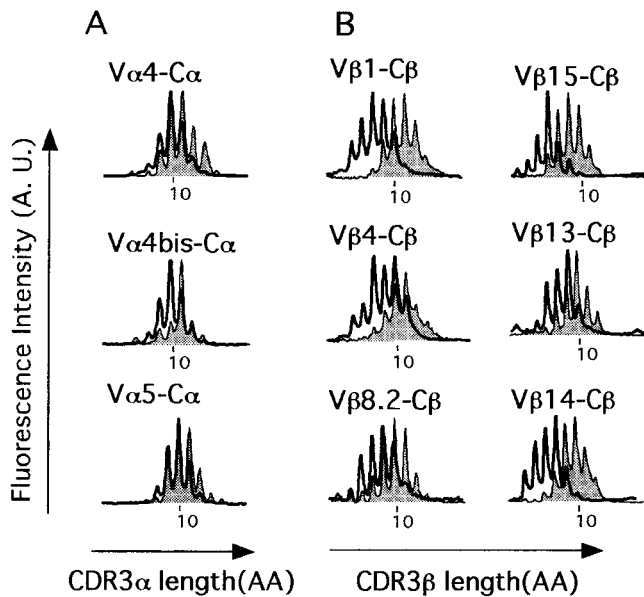


Figure 1. CDR3 length distributions of T lymphocytes. V α -C α (A) and V β -C β (B) from C57Bl/6 (shaded gray) and Tdt $^{\circ}/^{\circ}$ (black line) are displayed.

implies that the recombination machinery is likely to be prevalent in the determination of the CDR3 length, rather than a selection process favoring a CDR3 length of 9 and 10 AA, usually seen in Tdt $^{+}$ animals.

We have examined the participation of N nucleotide addition in the diversity of the TCRBV chain by estimating the size of the TCRBV chain repertoire in Tdt $^{\circ}/^{\circ}$ mice. We performed extensive sequencing of different BV-BJ combinations from Tdt $^{\circ}/^{\circ}$ spleen T cells. The results are summarized in Table I. We either sequenced a purified PCR band corresponding to a given CDR3 length (columns A and B) or a PCR band comprising all CDR3 lengths (columns C-F). We then estimated the maximum probable number of distinct CDR3 sequences present within an aliquot (MLE). The TCRBV chain repertoire is calculated according to the following equation: MLE of distinct sequences \times 1/% BV-BJ usage \times 1/% immunoscope peak area (this last parameter equals 1 when all CDR3 lengths are sequenced). For instance, for the 6 AA long CDR3 BV10-BJ1.2 rearrangement (column B, Table I), the MLE of distinct sequences is 17. As the percentage of T splenocytes using BV10-BJ1.2 is 0.15% (18, 19) and the 6 AA long CDR3 peak area represents 24.7% of the total BV10-BJ1.2 immunoscope profile, we estimate the number of distinct TCRBV chains present in the spleen of Tdt $^{\circ}/^{\circ}$ mice to be $(17 \times 100/0.15 \times 100/24.7) = 4.5 \times 10^4$. In another experiment, we sequenced the BV10-BJ1.2 PCR product encompassing all CDR3 lengths (column C, Table I). A MLE of 82 was obtained, giving rise to an estimated size of the TCRBV chain repertoire of 5.4×10^4 . The different BV-BJ pairs studied show reproducible values for the TCRBV repertoire size (between 2.7×10^4 and 5.4×10^4). Recently, our group (13) has estimated the

Table I. Estimate of the TCRBV Repertoire Size in Tdt $^{\circ}/^{\circ}$ Mice

Rearrangement	Unsorted splenocytes			Sorted splenocytes		
	A	B	C	D	E	F
	BV7-BJ1.2	BV10-BJ1.2	BV10-BJ1.2	BV6-BJ1.2	BV10-BJ1.2	BV10-BJ1.2
Studied CDR3 β length (AA)	9 ^b	6 ^b	all ^c	all ^c	all ^c	all ^c
Total no. of sequences performed	93	284	206	249	355	242
MLE of distinct sequences ^d	12	17	82	75	63	78
Studied BV-BJ $^{+}$ T cells (%) ^e	0.35	0.15	0.15	0.18	0.15	0.15
TCRBV size estimate ^f	2.7×10^4	4.5×10^4	5.4×10^4	4×10^4	4.2×10^4	5.2×10^4

In a previous report (13), we have shown that the BV repertoire is $4.7\text{--}6 \times 10^5$ in DBA/2 mice and 5.7×10^5 in C57Bl/6 mice.

^aIn column D and E, distinct sequences performed on AV2 $^{+}$ and AV2 $^{-}$ samples were combined to estimate the size of the BV repertoire.

^bThe percentage of BV-BJ rearrangements bearing the indicated CDR3 β length was calculated from the immunoscope profile. The 9 AA long BV7-BJ1.2 rearrangement represents 12.9% of the total immunoscope profile. The 6 AA long BV10-BJ1.2 rearrangement represents 24.7% of the total immunoscope profile. TCRBV size estimate was calculated as follows: MLE \times 1/% BV-BJ usage \times 1/% area peak.

^cAll CDR3 lengths of BV-BJ rearrangements are sequenced.

^dMLE was calculated as described (reference 13).

^ePercentage of BV6 and BV10 usage among T splenocytes was determined by flow cytometry. The BJ1.2 usage was derived from Kato et al. (reference 18) and Candeias et al. (reference 19). By real time quantitative PCR, we determined that the BJ1.2 usage is similar in both strains: $5.5 \pm 1.02\%$ in C57Bl/6 and $6.7 \pm 1.7\%$ in Tdt $^{\circ}/^{\circ}$ mice (three animals per group).

^fTCRBV size estimate was calculated as follows: MLE \times 1/% BV-BJ usage.

number of distinct TCRBV chains in normal mouse splenocytes to be between 4.7 and 7×10^5 . Thus, the size of the TCRBV chain repertoire is decreased by a factor of 10 in Tdt^{o/o} mice compared with C57Bl/6 mice.

To determine the impact of memory T cells on the BV repertoire of Tdt^{o/o} mice, we compared the TCRBV diversity in T splenocytes depleted of memory T lymphocytes to unfractionated T splenocytes. Memory T cells are characterized by the CD44^{high}CD62L⁻ phenotype (20). Thus, a suspension of T cells from a single Tdt^{o/o} spleen was split into two aliquots. One was unfractionated while T cells from the other were sorted out according to the TCR⁺CD44^{intermediate} CD62L⁺ phenotype. The TCRBV chain repertoire size was determined in both samples. Data presented in Table I (columns C and F) show that the estimated TCRBV repertoire size is not significantly different in unsorted splenocytes and in CD44^{intermediate}CD62L⁺ splenocytes (5.4×10^4 versus 5.2×10^4). Thus, the repertoire of CD44^{high}CD62L⁻ T cells, presumably associated with a memory phenotype, has no significant influence on the estimate of TCRBV repertoire size (at least in SPF mice).

Given the large numbers of TCRAV and TCRAJ segments and the absence of information on the AJ frequency, quantification of TCRAV chain repertoire size in mice had not been achieved until the present work. Thus, we first measured the frequency of the AJ44 gene segment in the T cell population from three Tdt^{o/o} and 3 C57Bl/6 mice. By real time PCR, we amplified AV2-AJ44 and AV2-AC rearrangements which allowed us to estimate the usage of AJ44. We obtained an average value of $4.7 \pm 1.1\%$. We then performed AV2-AJ44 and AV8-AJ44 PCR amplification on T splenocyte cDNA from Tdt^{o/o} and wt mice. MLE of distinct sequences were calculated (Table II). As

Table II. Estimate of TCRAV Chain Repertoire Size in C57Bl/6 and Tdt^{o/o} Mice

Rearrangement	AV2-AJ44		AV8-AJ44	
	C57Bl/6	Tdt ^{o/o}	C57Bl/6	Tdt ^{o/o}
Background				
CDR3 α size (AA)	all	all	all	all
Total no. of sequences performed	128	157	72	23
MLE of distinct sequences	72	17	22	9
Studied AV-AJ ⁺ T cells (%) ^a	0.61	0.61	0.18	0.18
TCRAV size estimate ^b	1.18×10^4	2.8×10^3	1.21×10^4	5.0×10^3

^aPercentages of AV2 and AV8 usage among T splenocytes were determined by flow cytometry. Percentages of AJ44 usage among T splenocytes were determined by real-time PCR as described in Materials and Methods.

^bTCRAV size estimate was calculated as follows: MLE \times 1/% AV-AJ usage.

the AV2⁺ T cell population represents 13% of the total pool of T cells (as determined by flow cytometry) and as $\sim 4.7\%$ of the AV2 chains bear the AJ44 segment, we estimated that the TCRAV chain repertoire size is 1.18×10^4 and 2.8×10^3 in Tdt⁺ and Tdt^{o/o} mice, respectively. Thus, the TCRAV chain repertoire in Tdt^{o/o} mice is reduced to $\sim 30\%$ of the C57Bl/6 TCRAV chain repertoire. In both strains of mice, despite a greater number of available gene segments, TCRAV diversity is one order of magnitude lower than the TCRBV diversity (5.7×10^5 TCRBV vs. 1.2×10^4 TCRAV in C57Bl/6, and 4.3×10^4 TCRBV vs. 2.8×10^3 TCRAV in Tdt^{o/o} mice).

Our group has recently shown that a unique TCRBV chain could associate on average with 2 or 3 TCRAV chains in the mouse (13) and with 25 in humans (12). One could hypothesize that the loss of diversity in the TCRAV and BV chains could be counterbalanced by an increase in α/β pairing, leading to a comparable level of TCR α/β repertoire diversity in Tdt^{o/o} and wt mice. To test this assumption, we sorted out AV2⁺ T cells from Tdt^{o/o} spleen. We sequenced all CDR3 lengths of two different BV-BJ rearrangements from AV2⁺ and AV2⁻ splenocytes. Results are summarized in Table III. MLE of 22 and 27 were obtained for BV6-BJ1.2 and BV10-BJ1.2 rearrangements, respectively. We estimate at 0.93×10^5 and 1.42×10^5 , respectively, the minimal number of different TCRs present in the spleen of Tdt^{o/o} animals at any given time. Thus, the TCR α/β repertoire in Tdt^{o/o} is decreased 10 to 20 times when compared with Tdt⁺ animals. Furthermore, as the TCRBV chain repertoire size is 4×10^4 and 4.2×10^4 (Table I, columns D and E), we conclude that one BV chain can associate on average with 2 to 4 different AV chains, as in normal mice (13). Thus, there is no increase in

Table III. Estimate of TCR α/β Repertoire Size in Tdt^{o/o} Mice

Rearrangement	BV6-BJ1.2	BV10-BJ1.2
	AV2 ⁺	AV2 ⁺
Sorted populations		
Studied BV-BJ ⁺ T cells (%) ^a	0.18	0.15
AV2 ⁺ T cells (%) ^b	13	13
MLE of distinct sequences	22	27
TCRBV size estimate ^c	1.2×10^4	1.8×10^4
TCR α/β size estimate ^d	0.93×10^5	1.42×10^5

^aPercentages of BV6 and BV10 usage among T splenocytes was determined by flow cytometry. The BJ1.2 usage was derived from Kato et al. (reference 18) and Candeias et al. (reference 19). By real time quantitative PCR, we determined that the BJ1.2 usage is similar in both strains: $5.5 \pm 1.02\%$ in C57Bl/6 and $6.7 \pm 1.7\%$ in Tdt^{o/o} mice (three animals per group).

^bPercentage of AV2 usage among T splenocytes was determined by flow cytometry.

^cTCRBV size estimate was calculated as followed: MLE \times 1/% BV-BJ usage.

^dTCR α/β size estimate was calculated as followed: MLE \times 1/% BV-BJ usage \times 1/% AV2 usage.

α/β pairing in $Tdt^{\circ/\circ}$ mice to counterbalance the decrease in diversity.

In this work, we have established that in $Tdt^{\circ/\circ}$ mice, the overall α/β T cell repertoire is only 5 to 10% of the wt repertoire. This means that between 100 to 200 T cells bear the same TCR (2×10^7 T splenocytes divided by 10^5 distinct TCRs). Does this suggest that they arose from the same precursor? Gilfillan et al. (21) showed that the positive selection of thymocytes is facilitated in $Tdt^{\circ/\circ}$ mice. However, the division rates of double positive as well as single positive thymocytes, measured by BrdU incorporation, are identical in $Tdt^{\circ/\circ}$ and wt mice. Thus, the number of identical mature thymocytes leaving the thymus is probably the same in both animals. We favor the hypothesis that the probability of generating the same rearrangements is greatly enhanced in $Tdt^{\circ/\circ}$ mice to explain the apparent large number of T cells bearing the same TCR.

These data represent the first estimate of the AV size repertoire in mice. Strikingly, the AV repertoire diversity is only one tenth of the BV chain diversity while in man they differ by a factor of two only (12). This difference between the ratios of BV diversity/AV diversity in man and mouse may be due to the recombination machinery itself or to a limitation in cell expansion during thymocyte development. In the thymus, double-negative thymocytes begin to rearrange their β -chains and divide before rearranging their α chains. In man, the number of cell divisions between the two rearranging events appears to be bigger (22) than in mice where it is ~ 6 to 7 (23), thus allowing the emergence of a larger number of distinct AV chains.

The functional importance of Tdt is attested by its presence in many vertebrate species but the physiological role of so much Tdt-related diversity remains to be shown. In this respect, Gavin et al. (11) showed that TCR from $Tdt^{\circ/\circ}$ mice were more cross-reactive than those from Tdt^+ animals, and suggested that such a polyreactive repertoire could increase the susceptibility of $Tdt^{\circ/\circ}$ animals to autoimmune diseases. However, (NZB \times NZW) F1 mice crossed on the $Tdt^{\circ/\circ}$ background are less susceptible to autoimmune nephritis than Tdt^+ mice with the same genetic background (24). Furthermore, when the Tdt null mutation was put onto the nonobese diabetic (NOD) background, it had a protective effect against insulinitis and diabetes (7). As Tdt-dependent repertoire appears to contribute to autoimmune diseases, it may be involved in resistance to as yet uncharacterized infectious diseases.

As mentioned $Tdt^{\circ/\circ}$ mice produce efficient immune responses. A minimal hypothesis to explain such observations would be that they use a marginally reduced repertoire. Non-mutually exclusive compensatory mechanisms could include an increased production of distinct germinal rearrangements and/or rearrangements with P diversity, an augmentation in α/β pairing. Our results are strikingly different from such hypotheses and show that the repertoire α/β size in $Tdt^{\circ/\circ}$ mice is only 5 to 10% of a normal repertoire, so none of the above compensatory mechanisms appear to be at work. Interestingly, the absolute number of rearrangements containing P nucleotides is comparable in

$Tdt^{\circ/\circ}$ and Tdt^+ animals (data not shown). Allelic exclusion of AV chains was tested with monoclonal antibodies against AV2, AV8, and AV11, less than 0.3% of cells express two different AV chains (data not shown). Moreover, no significant difference was found in allelic exclusion of AV chains between both strains of mice.

Is diversity important for the survival of a given species? When we compared the CDR3 β sequences in different $Tdt^{\circ/\circ}$ animals, we found that recurrent sequences represent between 20 and 25% of total sequences, showing that extensive variability exists between different $Tdt^{\circ/\circ}$ individuals. A similar comparison was made in Tdt^+ mice, the percentages of recurrent CDR3 β sequences were ~ 6 to 17%, depending on the CDR3 β lengths (13, 25). Thus, the higher diversity of Tdt^+ repertoire may be useful at the population level and should be tested in comparing the survival of $Tdt^{\circ/\circ}$ and Tdt^+ mice infected with highly mutagenic pathogens or in the wild.

It is worth emphasizing that if a T cell repertoire size decreased by a factor of 10 to 20 still protects $Tdt^{\circ/\circ}$ efficiently, thus the enormous diversity, calculated by Davis and Bjorkman (26), might not be needed. Langman and Cohn's hypothesis (27) that a functional repertoire contains $\sim 10^5$ distinct B lymphocytes (i.e. B cell protecton) must be considered as an alternative for T lymphocyte repertoire.

Several groups have shown that secondary T cell responses use a less diverse repertoire than primary responses (28–30). This is due to the preferential selection and expansion of T cells bearing the highest affinity TCR. It is conceivable that the decrease in $Tdt^{\circ/\circ}$ T cell repertoire diversity leads to a less efficient affinity maturation process. However, one can argue that $Tdt^{\circ/\circ}$ T lymphocytes may overcome this problem by upregulating their coreceptors and thus increase their overall avidity for antigen in secondary responses.

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