Age-Related Dysregulation in CD8 T Cell Homeostasis: Kinetics of a Diversity Loss

Joël LeMaoult, Ilhem Messaoudi, John S. Manavalan, Heather Potvin, Dragana Nikolich-Zugich, Ruben Dyall, Paul Szabo, Marc E. Weksler and Janko Nikolich-Zugich

J Immunol 2000; 165:2367-2373; doi: 10.4049/jimmunol.165.5.2367
http://www.jimmunol.org/content/165/5/2367

References
This article cites 12 articles, 7 of which you can access for free at:
http://www.jimmunol.org/content/165/5/2367.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Age-Related Dysregulation in CD8 T Cell Homeostasis: Kinetics of a Diversity Loss

Joël LeMaoult,* Ilhem Messaoudi,*‡ John S. Manavalan,† Heather Potvin,†
Dragana Nikolich-Zugich,* Ruben Dyall,* Paul Szabo,† Marc E. Weksler,‡‡ and
Janko Nikolich-Zugich2*,†‡

Relative diversity and representation of peripheral T cells bearing different TCR Vβ families are remarkably tightly regulated between birth and advanced adulthood. By contrast, individual elderly humans and C3H.SW and B10.BR aged mice display drastic disruption in such regulation. It was suggested that the alterations in the murine aged T cell compartment were due to age-related clonal T cell expansions (TCE). Here, we studied the kinetics of homeostatic dysregulation of T cell populations in aged C57BL/6 (B6) mice. Using mAb staining, we show that the percentages of αβ⁺CD8⁺ or CD4⁺ T cells bearing different TCRVβ elements remain virtually constant in mice up to 12 mo of age. In 22-mo-old mice, however, there is a dramatic disturbance of this pattern owing to the emergence of CD8⁺ TCE. Expanded T cells did not show any obvious bias in Vβ usage and were derived in all cases examined thus far from a single clone. TCE appeared later in life, compared with B cell clonal expansions. However, and in contrast to those detected in humans, TCE were frequently unstable disappearing within 2–4 mo, with other TCE appearing within the same time frame. Additional studies carried on thymic T cells, thymectomized mice, and young T transferred cells into Rag1⁻/⁻ mice suggest that the clonal expansions occur in the periphery and that their onset is accelerated by decreased thymic output and/or function(s). The Journal of Immunology, 2000, 165: 2367–2373.

Copyright © 2000 by The American Association of Immunologists 0022-1767/00/$02.00

Received for publication April 14, 2000. Accepted for publication June 14, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by U.S. Public Health Service Grant P01 AG-14669 from the National Institutes of Health (M.E.W.; J.N.-Z.); Memorial Sloan-Kettering Cancer Center Core Support Grant CA-08253, the Wright Chair in Geriatric Research (M.E.W.), and the DeWitt Wallace Fund (J.N.-Z.).

2 Address correspondence and reprint requests to Dr. Janko Nikolich-Zugich, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 98, New York, NY 10021. E-mail address: nikolicj@mskcc.org

3 Abbreviations used in this paper: TCE, T cell clonal expansion; CDR3, complementarity-determining region 3; TCRB, rearranged β gene of the TCR; FCM, flow cytfluorometric.
Laboratory (Bar Harbor, ME), and MHC class I knockout mice were purchased from Taconic Farms (Germantown, NY).

Adult thymectomy, partial splenectomy

Adult thymectomy was performed as described (6, 7), except that vacuum suction was applied to remove the organ. Partial splenectomy was performed on 18-mo-old mice as described (8, 9).

Monoclonal Abs and flow cyt fluorometric (FCM) analysis

For surface staining, the F23.1 mAb (anti-Vβ8.1/8.2/8.3) was purified and conjugated to FITC in our laboratory. FITC-conjugated anti-TCRβ; anti-Vβ2, 4, 5, 6, 8.3, 9, 10, 11, 13, 14; PE-conjugated anti-CD49d; and FITC- and APC-conjugated anti-CD62L mAbs were purchased from PharMingen (San Diego, CA). FITC-conjugated anti-Vβ 7, 12; anti PE-conjugated anti-CD4; and Tri-Color-conjugated anti-CD8 mAbs were from Caltag (San Francisco, CA). Analysis was performed on a FACSscan instrument using CellQuest 3.1 software (Becton Dickinson, Mountain View, CA). At least 10⁵ cells were acquired per sample. Dead cells were excluded by selective scatter gating.

DNA and cDNA samples

Total RNA was extracted using the RNA-Isolator Kit (Genosys Biotechnologies, The Woodlands, TX) and quantitated by OD. For cDNA synthesis, up to 5 μg of total RNA were reverse transcribed using the avian myeloblastosis virus-reverse transcriptase kit (Boehringer Mannheim, Indianapolis, IN) and oligo(dT)₁₅ primers in a 30-m reaction. After the reaction was completed, 30 μl double-distilled water were added.

Oligonucleotides

Oligonucleotides specific for the murine Vβ families (BV primers) and for the Cβ genes (BC primers), as well as the 5-carboxylfluorescein (FAM), 6-carboxy-X-rhodamine, or 6-carboxy-N,N,N,N'-tetrarmethylrhodamine-labeled BC_RO primers (BC_RO_X) are listed in Table I and Ref. 10.

PCR, runoff, and data analysis for TCRβ chain CDR3 length analysis

PCR and runoff reactions were performed as previously described (11). Briefly, classical PCR were performed on 2–5 μl cDNA in 25 μl with a sense primer specific for a BV family and an antisense primer specific for the constant BC region (BV and BC1 primers). For these PCRs, we used 0.2 μM concentrations of each primer, 1.5 mM MgCl₂, and 1 U Taq DNA polymerase (Perkin-Elmer, Roche Molecular Systems, Branchburg, NJ). The PCR products were visualized on a 1% agarose gel by ethidium bromide staining. Two microliters of the BV-BC1 PCR products were then labeled by 10 cycles of runoff elongation using the fluorescent BC_RO_X primers. Runoff elongation products, mixed with the same volume of 95% (v/v) formamide/10 mM EDTA, were run on a 6% acrylamide sequencing gel (FMC Bioproducts, Rockland, ME) loaded on an automated ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA). 6-Carboxy-X-rhodamine-labeled size markers GS350 (Applied Biosystems, Foster City, CA) were also loaded. Size determination of the runoff products and CDR3 size analysis was performed with Immuno sofware (11).

Sequencing

For sequencing, BV-BC1 PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). One-fourth of the purified PCR products were then sequenced using the nested BC3 primer or the appropriate BV primer and the BigDye Terminator sequencing kit (Applied Biosystems). Nucleotides contributed by the BV, BD, or BJ genes were identified by alignment with germline gene sequences (12).

Results

Kinetics of a homeostatic disturbance in relative Vβ representation among CD8ʰ⁺ T cells

For these studies on the influence of age on T cell subpopulation percentages, we chose to follow 1) TCRVβ protein expression using all available TCRVβ-specific mAbs and 2) diversity at the level of Tcr-β gene rearrangement using TCRBV CDR3 length analysis. Thus, the terms Vβ and BV will be used for the protein and gene, respectively. Spleen cells of 5 individual mice aged 3, 6, 9, 12, and 24 mo were triple-stained with anti-CD4, anti-CD8, and with each of the available anti-Vβ mAbs. As described before, in 3-mo-old animals (1, 3), the proportions of CD4⁺ or CD8⁺ T cells expressing a particular Vβ element seem to be tightly regulated, because they did not differ from one animal to the other. The same was true of 6-, 9-, and 12-mo-old mice. In addition, no differences in the proportions of CD4⁺ or CD8⁺ T cells expressing a particular Vβ element were detected when mice of different age groups were compared.
It has been previously reported that in old C3H.SW and B10.BR animals (3), proportions of T cell subpopulations expressing a particular Vβ element vary considerably between animals. We studied more than thirty 22-mo-old C57BL/6 mice. Similarly to findings published for the C3H.SW and B10.BR mouse strains, we observed expansions of some T cell subpopulations, sometimes to dramatic proportions. These expansions involved the CD8\textsuperscript{+} but not the CD4\textsuperscript{+} T cell subset. CD8\textsuperscript{+} TCE did not seem to exhibit any bias in Vβ element utilization, suggesting that they were equally recruited from among T cells expressing all TCRVβ families. Fig. 1 shows representative FCM results obtained for the CD8\textsuperscript{+} subset of two mice younger than 12 mo and three 22-month-old mice.

**Alterations in the percentages of CD8\textsuperscript{+} T cells expressing particular Vβ elements are due to clonal expansions**

Total RNA was extracted from the samples analyzed by FACS as above, and CDR3 length analysis was performed for every functional BV gene, to evaluate the diversity of the quantitatively expanded and nonexpanded T cell populations. We found a strong correlation between alteration of the percentages of CD8\textsuperscript{+} T cells expressing a particular BV gene and alteration of the TCRBV CDR3 length analysis patterns for this same population. Indeed, in mice up to 12 mo of age, the CDR3 length analysis patterns were bell-shaped (i.e., “polyclonal” in Fig. 2A, Box 1) and indistinguishable regardless of age. This strongly suggests that the TCR diversity of T cells remains unaltered between the ages of 3 and 12 mo. This apparent stability of the overall TCR repertoire at the molecular level correlates tightly with the stability of the percentages of T cells expressing a particular Vβ segment seen in Fig. 1.

When TCRBV CDR3 length analysis was carried on the same thirty 22-month-old mice as above, two types of CDR3 length patterns were noted. Although many CDR3 length patterns were unchanged as compared with the 3–12-month-old controls, others exhibited significant changes, sometimes of dramatic proportions (Fig. 2A, boxes 2 and 3). Representative results for a 22-mo-old mouse are presented in Fig. 2B. The alterations in TCRBV CDR3 length patterns were of two magnitudes: 1) small alterations, with losses of the characteristic bell shape but clear maintenance of the other parameters indicating relative CDR3 length diversity (Fig. 2B, BV2 and BV12); and 2) dramatic alterations, with the detection of only one CDR3 length (Fig. 2B, BV10). Invariably, alterations of the CDR3 length patterns happened in the T cell populations expressing a particular Vβ protein for which the percentage of CD8\textsuperscript{+} T cells was altered by mAb staining (Fig. 2C). T cell populations bearing Vβ families that exhibited only slight CD8\textsuperscript{+} TCE expansions were also those for which only small alterations of the TCRBV CDR3 length patterns were detected (small TCE). By contrast, large expansions of the percentages of CD8\textsuperscript{+} T cells expressing a particular TCRBV protein corresponded to the most dramatic alterations of the CDR3 length patterns. In cases where only one CDR3 length pattern was detected, large expansions of the percentages of CD8\textsuperscript{+} T cells were sequenced using the BV-BC PCR product as a template. All such PCR products yielded clear and unambiguous CDR3 sequences, indicating that the great majority of the BV-BC PCR product was made up of only one sequence (data not shown). This indicates that the expansions detected by mAb staining were due to the expansion of clonal T cells. Furthermore, we conclude that there is precise correlation between the TCE detected by FCM and the appearance of expanded clones detected by TCRBV CDR3 length analysis at the molecular level.

**The frequency of age-related T cell clonal expansions increases between the ages of 18 and 25 mo**

To further investigate the kinetics of appearance and the stability of TCE, we followed a set of eight mice over time using TCRBV CDR3 length analysis of PBLs at 18, 20, 22, 24, and 25 mo. Analysis was performed for all functional BV genes at 18 and 25 mo of age and for BV4, BV5, BV6, BV8S2, and BV10 at 20, 22, and 24 mo of age. At 18 mo of age, only two mice showed TCE, none of them in BV4, BV5, BV6, BV8S2, or BV10. For the later time points, however, such clonal expansions could be detected with increasing frequency (Table II). Consequently, at the age of 25 mo, 60% of the mice had developed at least one TCE (study conducted...
Mechanism of TCE appearance

To determine whether the T cell clonal expansions detected in the periphery originated from the thymus or from peripheral cells, we compared the CDR3 length profiles of PBLs and of the thymic cells of 20-mo-old animals. Although peripheral TCE were clearly unstable, particularly in early senescence, although frequent and sometimes dramatic, are frequently fluid and unpredictable with respect to Vβ usage (e.g., Vβ5 and Vβ12), many newly arising TCE were distinct from one another, and the Vβ utilization did not correlate in any obvious fashion to the age of the donor. These results indicate that the age of the T cells themselves is not necessarily predictive of their propensity to produce TCE. Rather, the number of divisions that the transferred T cells have undergone to fill the empty lymphocyte compartment of the recipient might be responsible for the development of T cell clonal expansions. After they transferred peripheral T cells from old mice with clonal expansions and from young control mice into Rag1-deficient animals, devoid of lymphocytes. Six months after transfer, PBLs were collected, CDR3 length analysis was performed, and the profiles before and after transfer were compared (Fig. 4, Old Donor). Of 20 attempted transfers, only one T cell clone (5% frequency) could be transferred successfully (not shown). All other T cell clones had completely disappeared, indicating that such T cell clonal expansions were of the “transient” type described above, that such T cell clones grow much more slowly than other T cells that are not expanded, or that their growth in the donor mouse was driven/supported by elements not transferred to the Rag1-deficient recipients. The most interesting finding, however, came from the fact that 1) although the T cell clones present in the donor mice had disappeared 6 mo after transfer (Fig. 4, lower panel, BV10 and, to a lesser extent BV20), new clonal expansions had arisen (Fig. 4, Old Donor, BV9 and BV15), and that 2) T cell clonal expansions had developed in a similar fashion in the recipients that had received PBLs from young control mice (Fig. 4, upper panel, BV9 and BV20).

Even when the same TCE-containing cell population was transferred into two recipients, the TCE arising were not necessarily identical. Although slight preferences were observed with regard to Vβ usage (e.g., Vβ5 and Vβ12), many newly arising TCE were distinct from one another, and the Vβ utilization did not correlate in any obvious fashion to the age of the donor. These results indicate that the age of the T cells themselves is not necessarily predictive of their propensity to produce TCE. Rather, the number of divisions that the transferred T cells have undergone to fill the empty lymphocyte compartment of the recipient might be responsible for the development of T cell clonal expansions. After they

Table II. Kinetics of age-related T cell clonal expansions between the ages of 18 and 25 mo for five Vβ families

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Mice with TCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>0/8</td>
</tr>
<tr>
<td>20</td>
<td>2/8</td>
</tr>
<tr>
<td>22</td>
<td>2/8</td>
</tr>
<tr>
<td>24</td>
<td>1/8</td>
</tr>
<tr>
<td>25</td>
<td>4/8</td>
</tr>
</tbody>
</table>

* PBLs from eight C57BL/6 mice were isolated sequentially at the ages of 18, 20, 22, 24, and 25 mo, and CDR3 length analysis was performed as described in Materials and Methods for BV4, BV5, BV6, BV8S2, and BV10. For each time point, the number of mice with at least one age-related T cell clonal expansion in one of the Vβ families studied was determined. Only clonal expansions large enough to bias the CDR3 length patterns so that only a single CDR3 length was detected qualified as age-related clonal expansions.
have repopulated the T cell compartment of the recipient, the transferred T cells have undergone a number of divisions compared with which the original differences in cell division history between the young and old donor T cells must be negligible. This might explain why transferred T cells from old mice did not give rise to more TCE in the recipient than T cells from young mice. This hypothesis is further strengthened by the fact that 18-mo-old mice that had been thymectomized at the age of 6 wk develop T cell clones twice as often as euthymic 18-month-old animals (J. LeMaoult, R. Dyall, and J. Nikolich-Zugich, unpublished observations).

Discussion

To further investigate the age-related disturbance in T cell homeostasis, we took advantage of our capacity to study the diversity of the T cell repertoire at several levels, by associating mAb staining with TCRβ-chain CDR3 length analysis and sequencing. We show that clonal expansions can be detected by mAb staining, extending the findings on the existence of CD8+ TCE bearing a particular V element to the C57BL/6 strain (3). Kinetic analyses show that these expansions are not detected before the age of 12 mo in these animals, and never in the CD4+ T cell subset. More importantly, using CDR3 length analysis in parallel with mAb staining, we undoubtedly established the clonal nature of the age-dependent T cell expansions. The reason for which TCE develop mainly in the CD8+ subset remains unclear; our studies on MHC class I-deficient mice showed that CD4+ TCE can develop, but studies carried on MHC class II-deficient mice confirmed that CD8+ T cell clones occur much more commonly (not shown).

CDR3 length analysis enabled us to carry longitudinal studies on the T cell clonal expansions previously identified by mAb staining, and we showed that age-dependent T cell clonal expansions are often transient. Over time, however, stable clonal expansions

<table>
<thead>
<tr>
<th>BV2</th>
<th>1</th>
<th>0</th>
<th>1</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV3</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td>BV5.2</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>BV9</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>BV11</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>BV12</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

Table III. Fluidity and stability of TCE over time in six BV families

A cohort of 60 mice was screened for TCE by CDR3 length analysis at 18 mo of age. Fourteen TCE were identified in the above 6 BV families. The same families were then followed 2 mo later in the same mouse cohort. TCE are defined by profile 3 (clonal) in Fig. 2A. Their clonal identity was confirmed by sequencing in 3 of 3 samples sequenced (confirming the absolute concordance of clonal CDR3 profiles and clonality by sequence in >100 independently analyzed cases in our laboratory thus far). N/A, not applicable.
or old mice with T cell clones were transferred i.v. into young Rag1−/− mice. Speeding the clearance of the pathogen. Such polyclonal immune responses would increase the efficiency and efficacy of an immune response, would allow numerous T cell clones to expand moderately and because of the availability of many relevant T cell clones with cognate Ags. These responses would go unnoticed in young animals just represent normal, although accentuated, responses against foreign Ags. These responses would go unnoticed in young animals just represent normal, although accentuated, responses against foreign Ags. Therefore, the relative age of the mouse is indicated by the top arrow. In situation A, a young animal with high thymic output and a high peripheral T cell diversity responds to antigenic challenge by recruiting many T cell clones of relevant and overlapping specificities. The response is efficient, and the T cell clones specific for the Ag need to expand only moderately and briefly before the pathogen is cleared. In situation B, an older organism, with low or no thymic output and decreased peripheral T cell diversity, faces antigenic challenge by recruiting a limited number of T cell clones specific for the Ag. The immune response is less efficient than in situation A, and each of the selected T cell clones must expand more and for a longer time than in situation A to clear the pathogen. During one of these expansions, one clone becomes insensitive to homeostatic mechanisms, persists, and forces these mechanisms to compensate, further decreasing the peripheral T cell diversity. In situation C, an old organism, with peripheral T cell clonal expansions and a severely decreased peripheral T cell repertoire diversity, responds to antigenic challenges by recruiting a very restricted set of T cell clones of relevant specificities. These must expand greatly, but even so, the quality of the response might be too poor to ensure a fast clearance of the pathogen. Consequently, the expansions last longer than in situation A or even B. In a worst case scenario, the limited set of recruited T cell clones might not succeed in clearing the pathogen.

The simplest explanation for the transitory skewing of the CDR3 length patterns, as well as for the transitory clonal expansions is that they are the consequences of a single cause and may just represent normal, although accentuated, responses against foreign Ags. These responses would go unnoticed in young animals because of the availability of many relevant T cell clones with overlapping specificities for a given pathogen. This redundancy would allow numerous T cell clones to expand moderately and would increase the efficiency and efficacy of an immune response, speeding the clearance of the pathogen. Such polyclonal immune responses would not necessarily significantly disturb the Vβ mAb staining profile, nor would they obligatorily skew CDR3 length patterns, especially against a very diverse background that would “buffer” the CDR3 length pattern variations (Fig. 5, situation A). The reason for which these benign immune responses would become TCE in an old animal is unclear. On the basis of our results, we propose that the T cell pool of such an animal is no longer diverse enough to select multiple T cell clones specific for the same pathogen. In such a “reduced diversity” system, the few T cell clones specific for the pathogen would need to expand more vigorously than in a young animal, in which more T cell clones can be recruited. Furthermore, in a reduced diversity system, T cells highly specific for the Ag might not be as available as in a diverse system. This would decrease the quality of the response, delay the clearance of the pathogen, and cause the selected T cells to multiply even more (Fig. 5, situations B and C). If this is the case in aged mice, it is not surprising that their peripheral T cell repertoire seems to be unstable; greater clonal expansions against a less diverse, less buffering background would show (transiently) until the pathogen is cleared. As for the stable TCE, we hypothesize that they arise from transient TCE by losing responsiveness to homeostatic control mechanism(s). We do not know whether the TCE we detected were made up of premalignant or malignant cells. However, preliminary data show that they do not divide actively, not even to the pace of other, nonexpanded peripheral T cells (not shown).

Even if the stably expanded T cells do not actively divide and may not be activated, their presence might have devastating consequences. To cite only one, such a large clonal T cell population (TCE), by its mere size, would force the homeostatic mechanisms to compensate for increased cellularity. Given that TCE no longer respond to such mechanisms, the activity of homeostatic mechanisms would predominantly affect (and down-regulate) non-TCE cells, further increasing the diversity losses (Fig. 5). Ultimately, such a deprived T cell pool is doomed to encounter a pathogen it cannot fight efficiently enough to ensure survival. Indeed, recent data from our laboratory confirm that TCE directly impact on the...
capacity of the immune system to efficiently respond to pathogens (J. LeMaoult, I. Messaoudi, B. M. Metzner, R. Rivi, K. Remus, M. E. Weksler, and J. Nikolich-Zugich, manuscript in preparation).

References