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Flow-Microfluorometric Monitoring of Oligoclonal CD8$^+$ T Cell Responses to an Immunodominant Moloney Leukemia Virus-Encoded Epitope In Vivo$^1$

Pierre Brawand,* Giovanni Biasi, † Clotilde Horvath,* Jean-Charles Cerottini* and H. Robson MacDonald$^{2*}$

The TCR repertoire of CD8$^+$ T cells specific for Moloney murine leukemia virus (M-MuLV)-associated Ags has been investigated in vitro and in vivo. Analysis of a large panel of established CD8$^+$ CTL clones specific for M-MuLV indicated an overwhelming bias for V$\beta$4 in BALB/c mice and for V$\beta$5.2 in C57BL/6 mice. These V$\beta$ biases were already detectable in mixed lymphocyte:tumor cell cultures established from virus-immune spleen cells. Furthermore, direct ex vivo analysis of PBL from BALB/c or C57BL/6 mice immunized with syngeneic M-MuLV-infected tumor cells revealed a dramatic increase in CD8$^+$ cells expressing V$\beta$4 or V$\beta$5.2, respectively. M-MuLV-specific CD8$^+$ cells with an activated (CD62L$^-$) phenotype persisted in blood of immunized mice for at least 2 mo, and exhibited decreased TCR and CD8 levels compared with their naive counterparts. In C57BL/6 mice, most M-MuLV-specific CD8$^+$ CTL clones and immune PBL coexpressed V$\alpha$3.2 in association with V$\beta$5.2. Moreover, these V$\beta$5.2$^+$V$\alpha$3.2$^+$ cells were shown to recognize the recently described H-2D$^b$-restricted epitope (CCLCTYVF) encoded in the leader sequence of the M-MuLV gag polyprotein. Collectively, our data demonstrate a highly restricted TCR repertoire in the CD8$^+$ T cell response to M-MuLV-associated Ags in vivo, and suggest the potential utility of flow-microfluorimetric analysis of V$\beta$ and V$\alpha$ expression in the diagnosis and monitoring of viral infections. The Journal of Immunology, 1998, 160: 1659–1665.

Although most examples of V$\beta$ (or V$\alpha$) bias in recognition of defined peptide:MHC complexes are based on sequencing of TCR from long-term established T cell clones, we (6) and others (7) have shown recently that a strong V$\beta$ (and/or V$\alpha$) bias can in some cases already be detected by flow microfluorometry during a primary immune response in vivo. Indeed, by immunizing normal DBA/2 mice with syngeneic (P815) tumor cells transfected with the human HLA-CW3 gene, we were able to detect a dramatic expansion of CD8$^+$V$\beta$10$^+$ T cells recognizing the decapeptide CW3 170–179 in association with H-2K$^d$ (6). At the peak of the HLA-CW3 response, CD8$^+$ T cells expressing V$\beta$10 were frequent in blood and lymphoid tissue of DBA/2 mice and were mainly restricted to a subset with an activated (CD62L$^-$CD44$^{high}$CD45RB$^{low}$) phenotype (6, 8).

It could be argued that murine CD8$^+$ T cell responses to a human HLA peptide expressed ectopically in transfected mastocytoma cells might represent an unusual (or even unphysiologic) model system. Therefore, we decided to investigate other well-characterized systems in which CD8$^+$ mouse T cells are known to respond to more physiologic Ags. Moloney murine leukemia virus (M-MuLV) is a retrovirus that readily infects newborn mice and leads to the development of T cell lymphomas. Most strains of adult mice, however, are able to establish long-lasting immunity to the virus and reject M-MuLV-infected cells. The response of adult C57BL/6 and BALB/c mice to M-MuLV has been particularly well studied (reviewed in Ref. 9). In these strains, CD8$^+$ T cell responses restricted by H-2D$^b$ and H-2K$^d$, respectively, have been shown to be protective. Moreover, the dominant epitope recognized by H-2D$^b$-restricted CTL in the response to the closely related Friend/Moloney/Rauscher (FMR) group of retroviruses has been identified very recently (10).

In the present study, we have determined the TCR repertoire of CD8$^+$ T cells in C57BL/6 or BALB/c mice responsive to

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$^1$ Abbreviations used in this paper: CDR, complementarity-determining region; CD62L, CD62 ligand; FMR, Friend/Moloney/Rauscher; MLTC, mixed lymphocyte: tumor cell culture; M-MuLV, Moloney murine leukemia virus; PEC, peritoneal exudate cell.

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M-MuLV Ags in vitro and in vivo. Our data indicate that there is a dramatic Vβ and/or Vα restriction in both strains, thereby strengthening the case for generalized oligoclonal CD8+ T cell responses to Ags and pathogens.

Materials and Methods

Mice and immunizations
C57BL/6 and BALB/c mice were obtained from HARLAN OLAC (Bicester, U.K.). M-MuLV-infected MBL-2 and LSTRA tumor cells were maintained by weekly passage in syngeneic C57BL/6 and BALB/c mice, respectively (11). For primary immunization, 50 × 10^6 irradiated (10,000 rad) tumor cells were injected i.p. into syngeneic mice. After 3 to 4 wk, secondary responses were elicited by i.p. injection of 10 × 10^6 viable syngeneic tumor cells. PBL and nylon wool-purified peritoneal exudate cells (PEC) were prepared as described previously (6).

Production of virus-immune and “carrier” mice
Cellfree preparations of Moloney murine sarcoma virus/M-MuLV complex obtained from progressing sarcomas induced in 2-wk-old C57BL/6 and BALB/c mice were used throughout this study. Adult (8–10 wk old) mice were injected i.m. in the thigh region with the syngeneic extract at a dose that had an Moloney murine sarcoma virus in vitro titer of 1 to 1.2 × 10^3 focus-forming U/ml on 3T3FL cells, and M-MuLV titer of 0.8 to 1 × 10^8 plaque-forming U/ml on SC-1 XC cells. These mice developed tumors that regressed in all instances within 14 days and were used as M-MuLV-immune spleen cell donors.

To obtain virus carrier mice (12), C57BL/6 and BALB/c mice were injected s.c., within 48 h after birth, with 0.05 ml of 0.1 gEq cellfree extract of a primary leukemia induced by M-MuLV in BALB/c mice. When these were mice of 10 to 12 wk old, they served as virus carrier spleen cell donors.

Mixed lymphocyte:tumor cell cultures and CTL clones

Virus-specific CTL were generated in vitro in a 5-day mixed leukocyte tumor cell culture (MLTC) (13). Briefly, 25 × 10^6 responder spleen cells from M-MuLV-immune mice and 5 × 10^6 irradiated leukemia (MBL-2 or LSTRA) or 2 × 10^5 stimulator spleen cells from carrier mice were cocultured in 15 ml of DMEM (Life Technologies, Paisley, U.K.) supplemented with 2 × 10^{-5} M glutamine, 2 × 10^{-5} M HEPES, 3 × 10^{-5} M 2-ME, antibiotics, and 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). Cells recovered from MLTC were washed and maintained for an additional 4 days in culture (2 × 10^6 cells/ml) in complete medium supplemented with 10 U/ml of rIL-2 (a generous gift of Sandoz, Basel, Switzerland) to permit the cytofluorimetric analysis of TCR Vβ expression. CTL clones were established by plating MLTC cells at limiting dilution, as described previously (14).

Cytotoxic assays

CTL clones or nylon wool-purified PEC from M-MuLV-immune mice were used as effector cells. Target cells were either MBL-2 lymphoma (M-MuLV infected, H-2b), RMA lymphoma (Rauscher virus infected, H-2b), or EL-4 lymphoma (FMR uninfected, H-2b). The FMR gag-encoded epitope CCLCLTVFL (10) was synthesized and purified by standard procedures and dissolved in DMSO. For cytotoxic assays, effector cells and 51Cr-labeled target cells were mixed at the indicated ratios in the presence or absence of various concentrations of peptide. Supernatants were harvested after 4 h, and specific 51Cr release was calculated as described previously (11).

Flow microfluorometry

At various times after primary or secondary immunization with syngeneic M-MuLV-infected tumor cells, C57BL/6 or BALB/c mice were bled by the tail vein and PBL isolated by Ficoll-Hypaque gradient centrifugation. Isolated PBL were routinely triple stained with mAbs against CD8 and Vα and Vβ described previously (14). In some experiments, four-color staining with mAbs to CD8, CD62L, and Vα anti-Vβ mAbs together with mAbs against CD8. To increase the sensitivity of such clones was remarkably restricted in both cases. In particular, 38 of 41 BALB/c clones utilized Vβ4, and 35 of 35 C57BL/6 clones used Vβ5. This Vβ preference was already clearly established among CD8+ T cells in MLTC from which the clones were derived (Table I). Thus, CD8+ Vβ4+ and CD8+ Vβ5+ cells were enriched by three- to fivefold in MLTC established from BALB/c and C57BL/6 mice, respectively (as compared with normal or immune spleen cells). Comparable results were obtained in mixed cultures using either irradiated virus-infected (carrier) spleen cells or tumor cells as stimulator cells (Table II), indicating that the selective Vβ expansion was specific for M-MuLV-associated (rather than tumor-specific) Ags. No significant Vβ-specific expansion of CD4+ T cells was observed in MLTC under the same conditions (Table II).

To investigate whether the observed in vitro Vβ bias in the CD8+ T cell repertoire to M-MuLV-associated Ags also occurs in vivo, C57BL/6 or BALB/c mice were injected with irradiated syngeneic M-MuLV-infected (MBL-2 or LSTRA) tumor cells and boosted with viable syngeneic tumor cells 2 to 4 wk later. This protocol has been shown previously to be optimal for the generation of M-MuLV-specific CTL (11, 14). PBL were pooled from immunized or control mice on day 10 and stained with a panel of anti-Vβ mAbs together with mAbs against CD8. To increase the sensitivity

Table I. Vβ repertoire of established CTL clones specific for M-MuLV-associated Ags

<table>
<thead>
<tr>
<th>Strain of Origin</th>
<th>Vβ4</th>
<th>Vβ5</th>
<th>Vβ6</th>
<th>Other Vβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>38</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

“CTL clones derived in MLTC (using either irradiated M-MuLV-infected tumor cells or irradiated spleen cells from M-MuLV-infected carrier mice as stimulators) were screened with a panel of anti-Vβ mAbs. Data for BALB/c and C57BL/6 mice are summarized for 11 and 4 independent MLTC cloning experiments, respectively.

Results

Preferential Vβ usage among CD8+ CTL clones and MLTC cells specific for M-MuLV-associated Ags

CD8+ CTL clones specific for M-MuLV-associated Ags can be generated readily in MLTC established from virus-immune BALB/c or C57BL/6 mice (11). Using a panel of anti-Vβ mAbs, we screened the Vβ repertoire of established M-MuLV-specific clones from these two strains. As shown in Table I, the Vβ usage of such clones was remarkably restricted in both cases. In particular, 38 of 41 BALB/c clones utilized Vβ4, and 35 of 35 C57BL/6 clones used Vβ5. This Vβ preference was already clearly established among CD8+ T cells in MLTC from which the clones were derived (Table I). Thus, CD8+ Vβ4+ and CD8+ Vβ5+ cells were enriched by three- to fivefold in MLTC established from BALB/c and C57BL/6 mice, respectively (as compared with normal or immune spleen cells). Comparable results were obtained in mixed cultures using either irradiated virus-infected (carrier) spleen cells or tumor cells as stimulator cells (Table II), indicating that the selective Vβ expansion was specific for M-MuLV-associated (rather than tumor-specific) Ags. No significant Vβ-specific expansion of CD4+ T cells was observed in MLTC under the same conditions (Table II).

Table II. Vβ bias among CD8+ T cells in MLTC restimulated with M-MuLV-associated Ags

<table>
<thead>
<tr>
<th>Strain of Origin</th>
<th>Vβ4</th>
<th>Vβ5</th>
<th>Vβ6</th>
<th>Other Vβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>38</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

“CTL clones derived in MLTC for 5 days with either irradiated syngeneic M-MuLV-infected tumor cells or irradiated syngeneic spleen cells from neonatally infected (carrier) mice. After further expansion in IL-2 for 4 days, cells were double stained with anti-Vβ mAbs and either anti-CD4 or anti-CD8 mAbs. Control spleen cells from normal and M-MuLV-immune mice were analyzed in parallel. Data represent mean ± SD of three to five experiments.
of detection of responding CD8\(^+\) cells, mAbs against CD62L (Mel-14) were included in the third color (8). As shown in Figure 1, PBL from C57BL/6 mice were highly enriched for V\(\beta\)5\(^+\) cells in the activated (CD62L\(^-\)) subset of CD8\(^+\) cells following secondary immunization with syngeneic MBL-2 tumor cells. In contrast, all other V\(\beta\) tested were utilized less frequently among CD62L\(^-\)CD8\(^+\) PBL. In PBL of BALB/c mice similarly immunized with M-MuLV-infected syngeneic LSTRA tumor cells, V\(\beta\)4\(^+\) cells were selectively enriched in the CD62L\(^-\)subset (Fig. 1). No V\(\beta\) bias was seen among CD62L\(^-\)CD4\(^+\)PBL in both strains (data not shown).

The V\(\beta\)5 gene family in the mouse is composed of two members (V\(\beta\)5.1 and V\(\beta\)5.2). To determine whether the V\(\beta\)5-biased anti-M-MuLV response in C57BL/6 mice involves both family members, we took advantage of the availability of a mAb that reacts selectively with V\(\beta\)5.1, but not V\(\beta\)5.2 (15). As shown in Figure 2, CD8\(^+\)CD62L\(^-\)PBL from M-MuLV-immunized C57BL/6 mice were highly enriched in cells that reacted with a pan anti-V\(\beta\)5 mAb, but were depleted of V\(\beta\)5.1\(^+\) cells. In contrast, CD8\(^+\)CD62L\(^-\)PBL from immunized mice contained the same proportion of cells reacting with both anti-V\(\beta\)5 mAbs as the equivalent population from unimmunized mice (Fig. 2). These data indicate that the CD8\(^+\) T cell response to M-MuLV in C57BL/6 mice is highly restricted to V\(\beta\)5.2\(^+\) cells. In accordance with these in vivo data, flow-microfluorometric analysis of the 35 V\(\beta\)5\(^+\)CTL clones described in Table I confirmed that 34 used V\(\beta\)5.2 (data not shown).

**Kinetics of the M-MuLV-specific immune response in C57BL/6 mice**

We also investigated the kinetics of appearance and persistence of V\(\beta\)5\(^+\)CD62L\(^-\) T cells in the peripheral blood of C57BL/6 mice following secondary immunization with syngeneic M-MuLV-infected MBL-2 tumor cells. As shown in Figure 3, the proportion of
Vβ5 cells was maximally elevated in the CD62L− subset of CD8+ PBL as early as 6 days after secondary immunization and persisted above background levels for at least 70 days. In contrast, no change in the proportion of Vβ5+ cells was observed at any time in the CD62L+CD8+ PBL subset. Although some variation in the proportion of Vβ5+CD62L−CD8+ PBL was seen between individual immunized mice, the overall evolution of the response was quite consistent (Fig. 3).

Down-regulation of TCR and CD8 in M-MuLV-immune T cells

We have shown recently that the CD62L− subset of CD8+Vβ10+ T cells in DBA/2 mice reactive with the H-2Kd-restricted epitope HLA-CW3 (170–179) expresses lower levels of CD8 and TCR when compared with their naive (CD62L+) counterparts (8). As shown in Figure 4, TCR and CD8 down-regulation is also apparent in the CD62L− subset of CD8+Vβ5+ T cells responding to M-MuLV-associated Ags in C57BL/6 mice. Quantitatively, the degree of reduction in TCR and CD8 expression (2–3 fold) is very similar in both antigenic systems, suggesting that down-regulation of both TCR and coreceptor is a common feature of Ag-specific activation of CD8+ cells in vivo.

Restricted Va usage by CD8+Vβ5+ M-MuLV-immune T cells

Since T cell clones specific for peptide:MHC complexes frequently exhibit restricted Va (as well as Vβ) usage, we also examined M-MuLV-immune PBL populations from C57BL/6 mice with the currently available mAbs against mouse Va domains. To maximize the sensitivity of this analysis, four-color staining was performed using mAbs against CD8, CD62L, and Vβ5 together with anti-Vα2, Vα3.2, Vα8, or Vα11 mAbs. As shown in Figure 5, CD8+CD62L−Vβ5+ PBL from M-MuLV-immune mice were highly enriched in cells expressing Vα3.2 (~70%), whereas the other three Va domains tested were under-represented. In contrast, CD8+CD62L+Vβ5+ PBL from immune or normal mice contained only a small subset (~5%) of cells that coexpressed Vα3.2, as expected from previous studies (16, 17).

Absolute magnitude of the M-MuLV-specific CD8+ T cell response

A major advantage of flow-microfluorometric monitoring of immune responses in vivo is that the absolute magnitude of the response can be readily determined (6, 7). Representative data for 15 individual M-MuLV-immune C57BL/6 mice measured at day 6 of the secondary response, as well as five unimmunized control mice, are summarized in Figure 6. On average, Vβ5+CD62L− cells accounted for 20% of the CD8 subset and 6% of total PBL in the immune mice. Interestingly, absolute numbers of Vα3.2+ cells were increased similarly in the CD62L−CD8+ subset (Fig. 6).
concerning the epitope(s) recognized by the major H-2Db-restricted
When this study was initiated, no information was available con-
gag
(CCLCLTVFL) encoded in the leader sequence of the M-MuLV
high proportion of specific CD8
not shown). Collectively, these data demonstrate that a remarkably
gag
predominantly recognize a dominant
FIGURE 6. Absolute magnitude of Vβ5- and/or
Va3.2-restricted CD8
T cell responses to M-MuLV. PBL from 15 M-MuLV-immune
C57BL/6 mice (day 6 after secondary immuniza-
and 5 control mice were stained in four colors
with mAbs against CD8, CD62L Vb5, and Va3.2
(see Fig. 5). The absolute proportion of CD62L
Vb5+ and/or Va3.2+ cells among total CD8+ cells
or total PBL is indicated for individual immune
(closed squares) or naive (open circles) mice. Bars
represent the logarithmic means of each group.

Most importantly, quantitation of CD8+CD62L− cells that coex-
press Vβ5 and Va3.2 resulted in absolute numbers that were al-
near CD8+ cells and 3.3% of total PBL). Similar calculations for Vβ5+
and/or Va3.2+ cells in the CD62L+CD8+ subset failed to dem-
strate any difference between naive and immunized mice (data
not shown). Collectively, these data demonstrate that a remarkably
high proportion of specific CD8+ T cells can be found circulating
in blood at the peak of the M-MuLV response. Moreover, they
indicate that coexpression of Vβ5 and Va3.2 by these immune
cells results in a significantly enhanced sensitivity of detection
when compared with either the Vb or Va domain alone (see
Discussion).

CD8+Vβ5+Va3.2+ M-MuLV-immune T cells and clones
predominantly recognize a dominant gag-encoded epitope
When this study was initiated, no information was available con-
cerning the epitope(s) recognized by the major H-2Db-restricted
CTL population responsible for rejection of M-MuLV-induced
tumors. However, Chen et al. (10) have shown recently that most
CTL specific for Ags encoded by the highly related FMR family of
retroviruses recognize a H-2Ddb-restricted nonapeptide
(CCLCLTVFL) encoded in the leader sequence of the gag
polyprotein. Since Vβ5+Va3.2+ cells dominate the CD8 T cell
response to M-MuLV, we therefore tested several Vβ5.2+Va3.2+ CTL clones for their ability to lyse EL-4 lymphoma cells (an H-2b tumor not infected by FMR retroviruses) in
the presence or absence of the gag peptide. As shown for a rep-
resentative CTL clone in Figure 7A, this peptide was efficient in
promoting lysis of EL-4 target cells in a dose-dependent fashion.
Furthermore, nylon wool-purified PEC isolated directly ex vivo
from M-MuLV-immune C57BL/6 mice at the peak of the response
also lysed EL-4 cells in the presence of the gag peptide (Fig. 7B).
Since peptide-dependent lysis of EL-4 cells by PEC was roughly
as efficient as lysis of RMA cells (a FMR
phoma cells (an H-2b tumor not infected by FMR retroviruses) in
in blood at the peak of the M-MuLV response. Moreover, they
indicate that coexpression of Vβ5 and Va3.2 by these immune
cells results in a significantly enhanced sensitivity of detection
when compared with either the Vβ or Va domain alone (see
Discussion).

FIGURE 7. Vβ5+Va3.2+ CTL clones and ex vivo M-MuLV-immune
CD8+ cells in C57BL/6 mice recognize an immunodominant gag-encoded
epitope. A, M-MuLV-specific CTL clones 6 (Vβ5.2+, Va3.2+, H-2Db-re-
stricted) or 464 (Vβ5+, Va3.2+, H-2Kb restricted) were tested for cyto-
Toxicity at an E:T ratio of 3:1 against EL-4 target cells (H-2b, FMR uninfected) in the presence or absence of various concentrations of the FMR
gag-encoded peptide CCLCLTVFL. B, Nylon wool-purified PEC from M-
MuLV-immune C57BL/6 mice (day 6 of secondary response) were tested for cyto-
toxicity against EL-4 target cells (FMR+) in the presence or absence of 1 μM gag-encoded peptide CCLCLTVFL. RMA target cells
(FMR+) served as a positive control. E:T cell ratios for PEC were also
calculated on the basis of Vβ5+CD62L−CD8+ cells (assessed by FACS
analysis).

Discussion
The data presented in this study demonstrate that the CD8+ T cell
response to M-MuLV-associated Ags in two independent mouse
strains is dominated by a highly restricted TCR repertoire. Thus,
CD8+ T cells from BALB/c and C57BL/6 mice preferentially use
Vβ4 and Vβ5.2, respectively, in response to M-MuLV. Moreover,
in the latter case, most Vβ5.2+CD8+ cells coexpress a single Va
element (Va3.2) and recognize an immunodominant epitope
(CCLCLTVFL) encoded in the leader sequence of the M-MuLV
gag polyprotein. Taken together with our previous data demon-
strating a Vβ10 (and to a much lesser extent Va8)-restricted re-
response to HLA-CW3 (6, 8, 18), it is thus tempting to speculate that
restricted TCR V gene usage is a common feature of CD8+ T cell
responses to Ag in vivo. Indeed, Vβ preferences also have been demonstrated recently in CD8+ T cell responses to acute infection by HIV (19), SIV (20), or EBV (21), and oligoclonality appears to be a general feature of the TCR repertoire of CD8+ (but not CD4+) cells in aged mice (22) and humans (23). Although the structural implications of such Vβ and Vα preferences remain to be fully elucidated, a minimal conclusion would be that the hypervariable CDR1 and/or CDR2 regions of the Vβ and Vα domains must play a role, either directly or indirectly, in the specificity of TCR binding to peptide:MHC complexes. Indeed, the recent resolution of the three-dimensional structure of two such trimolecular complexes indicates that (at least in certain cases) direct contacts between peptide residues and the CDR1 and/or CDR2 domains of the TCR can occur (2, 3).

A comparison of the phenotypic properties of Vβ-restricted anti-M-MuLV or anti-HLA-CW3 cells allows certain generalizations to be made. In both systems, the responding CD8+ cells appear to be specific for TCR and coreceptor, since a number of different Vβ populations based on single cell PCR sequencing (24). Whether a direct correlation between the response (25) and control (CD62L+APC) subset of cells expressing the appropriate Vβ domain. As discussed elsewhere (8), this down-regulation appears to be specific for TCR and coreceptor, since a number of other surface markers are not affected. Furthermore, cell size (as assessed by forward scatter) is very similar in both responding (CD62L+) and control (CD62L-) subsets, indicating that reduced TCR and CD8 expression is not related to decreased surface area. The functional consequences of TCR and CD8 down-regulation on immune T cells remain to be determined. Since CD8 appears to interact directly with the TCR upon ligation of peptide:MHC complexes (25, 26), one interesting possibility would be that a simultaneous reduction in TCR and CD8 density on immune cells would result in an overall increased threshold of activation. In this way, Ag-primed CD8+ T cells (already selected for a high affinity TCR) would be less susceptible to nonspecific activation by cross-reactive Ags.

Although Vβ-restricted CD8+ T cell responses to M-MuLV-associated Ags share many features with the anti-CW3 response, there is at least one important difference between the two systems. Whereas Vβ10+ CD8+ cells are already highly enriched in DBA/2 mice 2 wk after a primary injection of viable P815-CW3 tumor transfectants, Vβ-restricted anti-M-MuLV responses in either C57BL/6 or BALB/c mice are only readily detected after secondary immunization with syngeneic M-MuLV-infected tumor cells. Several explanations could account for this striking difference. First, it is possible that the frequency of CD8+ precursor cells specific for CW3 in DBA/2 mice is much higher than the anti-M-MuLV-specific precursor frequency triggered in the other strains. However, recent estimates using two independent approaches suggest that the frequency of specific precursors triggered in the CW3 model is in fact surprisingly low (15–20 per mouse) (18, 24), making it unlikely that the anti-M-MuLV precursor frequency could be much lower and still give rise to a reproducible secondary response. Alternatively, differences in the requirement for T cell help or in the expression of adhesion/costimulatory molecules by the immunizing tumor cells themselves could account for the more efficient primary response in the CW3 system. Experiments are currently in progress to attempt to distinguish among these various possibilities.

An important finding in the present study was the dramatic co-expression of Vβ5 and Vα3.2 on M-MuLV-specific CD8+ T cells in C57BL/6 mice. Although T cell responses restricted by both Vβ and Vα domains have been demonstrated previously in vivo for the CD4 response to cytochrome c (7), our data represent (to our knowledge) the first clear example for CD8+ cells. An obvious practical advantage of monitoring Vβ- and Vα-restricted T cell responses by flow microfluorometry is that the background values (in naive mice) are much lower than those found for responses restricted by Vβ or Vα alone. For example, since Vβ5+ and Vα3.2+ cells account for 15 and 5%, respectively, of the CD8 subset in naive mice, the threshold for detection of Vβ5+ Vα3.2+ “double-positive” CD8 cells in immune mice is reduced to 0.75% (assuming random association of α- and β-chains). This threshold reduction represents a gain in sensitivity of 10- to 20-fold compared with individual monitoring of Vβ and Vα domains.

In summary, our data provide strong evidence that a restricted TCR repertoire is a common feature of CD8+ T cell responses to physiologic Ags such as M-MuLV in vivo. In addition to their theoretical and structural implications, these findings suggest that Vβ and/or Vα repertoire screening of CD8+ PBL (particularly in conjunction with an appropriate activation marker) will prove to be an extremely useful tool in the diagnosis and monitoring of viral infection.

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References


