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PREDOMINANT UTILIZATION OF $V\beta_8^+$ T CELL RECEPTOR GENES IN THE H-2L^d-RESTRICTED CYTOTOXIC T CELL RESPONSE AGAINST THE IMMEDIATE-EARLY PROTEIN pp89 OF THE MURINE CYTOMEGALOVIRUS

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Cytotoxic T cell responses to the murine *Cytomegalovirus* (*MCMV*) were elicited in BALB/c mice (H-2^d) by infectious virus. Eight days after infection, *MCMV*-primed local lymph node T cells were either depleted for T cells expressing a $V\beta_8^+$ TCR or separated into $V\beta_8^+$ and $V\beta_8^-$ subpopulations by a cell sorter using the mAb F23.1. T cells were then expanded in vitro under limiting dilution conditions in the presence of IL-2 and in the absence of viral Ag to avoid selection by Ag in vitro. Frequencies of CTL precursors specific for the Immediate-Early-Ag 1 of *MCMV* and restricted to H-2 L^d were determined. L cells of the endogenous haplotype H-2^k cotransfected with the genes for *MCMV*-IE 1 and H-2L^d were used as target cells. Detection of a CTL response required previous priming of the animals by infection in vivo (<1/10^6 for nonimmunized animals). In primed animals CTL precursors of this specificity and restriction were three to fivefold more frequent in the $V\beta_8^+$ population (1/9.900 to 1/22.300) than in the $V\beta_8^-$ population (1/57.000 to 1/87.200). Control experiments showed that frequencies were not influenced by the treatment with the anti-$V\beta_8$-antibody and the fluorescein-labeled anti-Ig itself. $V\beta_8^+$ and $V\beta_8^-$ T cells did not reveal any frequency differences when several other responses were determined (TNP-specific self-restricted CTL precursor; Th cells specific for keyhole limpet hemocyanin or *Listeria monocytogenes*).

T cells recognize Ag in association with products of the MHC (1). The recognition is mediated by the Ag specific, MHC-restricted TCR (2, 3). The TCR is a heterodimer composed of α- and β-chains which are encoded by sets of variable (V, D, J) and constant (C) region genes (4). In mice, about 30 Vβ, 2 Dβ, 14 Jβ, and 2 Cβ genes code for the β-chain, and about 100 Vα, 20 Jα, and 1 Cα genes code for the α-chain (4). The diversity of the T cell repertoire is generated by rearrangement of TCR genes from their germ-line configuration (5). The $V\beta_8$ TCR-gene-family consists of three TCR genes ($V\beta_{8.1}$, $V\beta_{8.2}$, $V\beta_{8.3}$) (4). Determinants on these three β-chains are detected by the mAb F23.1 (6, 7). In BALB/c mice 30% of all peripheral T cells express $V\beta_8$ TCR genes (C57BL/6: 20%) (6). Given the estimates of 30 different Vβ genes, the $V\beta_8$ TCR genes are expressed more often than statistically expected. $V\beta_8$ TCR genes are expressed on both MHC class I- and class II-restricted T cells, respectively (6, 8).

Attempts to clarify the structure/function relationship of the TCR α- and β-chains with respect to their Ag specificity and/or MHC restriction were performed by a number of investigators mainly by the analysis of T cell clones and T cell hybridomas (9–17). With respect to the expression of $V\beta_8$ TCR genes, positive correlations were recently described for the T cell response against the autoantigen myelin basic protein causing autoimmune encephalomyelitis (18, 19) and for the susceptibility to collagen-induced arthritis in mice (20). A correlation of $V\beta_8^+$ TCR with I-E^d restriction was proposed from the analysis of sperm whale-specific, I-E^d-restricted T cell clones (17). $V\beta_{8.1}^+$ as well as $V\beta_6^+$ T cells were demonstrated to undergo negative thymic selection in Mls^a mice, suggesting high affinities between $V\beta_{8.1}^+$ or $V\beta_6^+$ TCR and Mls^a (21, 22). The general conclusion, however, appears to be that there are no simple rules according to which certain TCR genes recognize a particular antigenic epitope in conjunction with the appropriate MHC protein.

Correlations of TCR-Vβ-gene expression with functional specificities for MHC/Ag are best investigated by studying responses limited to the combination of a single antigenic epitope and a single MHC molecule. A response of this type arises in the infection of the immunocompetent BALB/c mouse with the *MCMV*[2] (23). Approximately 50% of all cytotoxic T lymphocytes sensitized in vivo by *MCMV* display specificity for IE gene products (24), which serve as viral regulatory proteins (25). About 25% of IE-specific CTL recognize the IE protein 1 (pp89) (26). This protein Ag is recognized by BALB/c CTL only in conjunction with H-2L^d (27). In adoptive transfer experiments into immunocompromised hosts, $CD8^+CD4^-$ T cells with specificity for pp89 were shown to effectively limit viral spread in tissues and to mediate protective immunity against lethal *MCMV* infection (28). Moreover, a vaccinia virus recombinant that expresses pp89 as the only *MCMV* protein elicits a $CD8^+$ T cell response that protects against lethal *MCMV* infection (29). Using deletion mutants as

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[2] Abbreviations used in this paper: *MCMV*, murine cytomegalovirus; CTL-p, CTL precursor; ConASN, Con A supernatant; IE, immediate-early; IE 1, IE Ag 1; ie 1, IE gene 1; KLH, keyhole limpet hemocyamin; LD, limiting dilution; pp89, immediate early 89 kDa phosphoprotein of *MCMV*.

well as synthetic peptides it was recently shown that a single T cell epitope is contained within a peptide spanning residues 161 to 179 of pp89 (27, 30).

We performed frequency analyses of T cells sensitized by *MCMV* in vivo, separated into $V\beta_8^+$ and $V\beta_8^-$ subpopulations, and expanded under limiting dilution conditions in vitro (23). Cultures were tested for cytotoxicity against transfected fibroblasts expressing IE-1 (pp89) and H-2L^d (26). These experiments provide evidence for a $V\beta_8$ TCR-gene-dominance in the H-2L^d-restricted CTL response against the regulatory viral nuclearprotein pp89 of the *MCMV*.

MATERIALS AND METHODS

Mice. C57BL/6, BALB/c, and C3H mice were bred in our animal facilities at the Max-Planck-Institute for Immunobiology, Freiburg, FRG, and used at the age of 8 to 10 wk.

Media. T cells were cultured in RPMI 1640 supplemented with 10% FCS (GIBCO, Grand Island, NY), 100 IU/ml penicillin (GIBCO), 100 μg/ml streptomycin (GIBCO), 10^{-5} M 2-ME (Merck, Darmstadt, FRG), 2 mM L-glutamine, and 25 mM HEPES buffer. Cells were cultured at 37°C in 10% CO_2/air.

Preparation of ConASN. Spleens from Sprague-Dawley rats were used for the preparation of ConASN as described earlier (31).

Antibodies. The mouse anti TCR antibody F23.1 (γ 2a, κ) recognizes $V\beta_{8.1,2,3}$ (6, 7) TCR gene products. The antibody was prepared from a hybridoma line kindly donated by Dr. U. Staerz, Basel, Switzerland, on Protein A-sepharose beads (Pharmacia, Freiburg, FRG) according to Ey et al. (32).

Ag. Cells were coupled with TNP as described earlier (33). KLH was obtained from Calbiochem, Frankfurt, FRG, NIP_{20}-KLH donated by Dr. B. Maier, Freiburg, FRG and *Listeria monocytogenes* by Dr. S. Kaufmann, Ulm, FRG. *MCMV* (strain Smith, VR-194) was obtained from the American Type Culture Collection, Rockville, MD and propagated on embryonic fibroblasts (23).

Immunizations. BALB/c mice were immunized by intraplantar infection with 2×10^5 plaque-forming units of *MCMV* in 100 μl PBS (23) or with 50 μg KLH in CFA as described (34). C57BL/6 mice were immunized with 5×10^4 live *L. monocytogenes* s.c. into the base of the tail.

T lymphocyte preparations. Primed T cells were derived from draining popliteal (*MCMV*) or inguinal (KLH, *L. monocytogenes*) lymphnodes 5 to 8 days after immunization and enriched by nylon wool passage.

Separation of $V\beta_8^+$ and $V\beta_8^-$ T cell subpopulations. T cells were incubated with F23.1 at a concentration of 5 μg/10^7 T cells for 30 min at 4°C, washed twice in RPMI 1640 containing 5% FCS, and stained with FITC labeled goat-anti-mouse-Ig F(ab')$_2$ (Dianova, Hamburg, FRG, 1/50) for 30 min at 4°C. Control samples were incubated with the FITC-coupled reagent only. Cells were washed three times, and transferred to PBS supplemented with 5% FCS (2×10^6 cells/ml). T cells were sorted on an Ortho Cytofluorograph 50 H (Ortho Diagnostic Systems, Raritan, N. J.), the sample tubing of which had previously been treated for 10 min with 0.5% potassium hypochloride solution (Javal, La Croix, Lesieur-Cotelle, Belgium) for sterilization. Gating conditions were set up to properly distinguish stained and unstained cells. Positively and negatively selected T cells were collected in sterile plastic tubes containing 1.5-ml culture medium. Viable cells were sorted at a flow rate of 1 to 1.5×10^3 gated events per second, and up to 4×10^6 cells were collected. To eliminate $V\beta_8^+$ T cells from total T cells, T cells were treated with F23.1 (20 μg/ml, 1 ml/2×10^7 cells) for 30 min at 4°C, washed, and incubated with rabbit C (diluted 1/10) (a kind gift of Dr. M. M. Simon, Freiburg, FRG) for another 30 min at 37°C. This treatment was repeated. All dilutions and washing steps were done in RPMI 1640. Successful depletion of $V\beta_8^+$ T cells was subsequently verified by flow microfluorimetry.

LD analysis. Frequencies of TNP-specific CTL-p were determined by LD analysis (33, 35). Briefly, T cells were serially diluted into microwells containing 3×10^5 syngeneic, X-irradiated, TNP-modified spleen cells, and cultured in the presence of 10% ConASN. Control cultures only received TNP-modified stimulator cells. After 7 days cytotoxicity of these cultures was tested against the tumor target cells RBL5 (H-2^b) with and without TNP modification.

Frequencies of CTL-p specific for *MCMV* IE 1 and restricted to H-2L^d were determined by LD analysis as previously described (26). Briefly, day 8 *MCMV* immune LN T cells were serially diluted and plated into microwells containing medium supplemented with 10% ConASN. In addition, each well received 3×10^5 syngeneic, nonimmune, X-irradiated spleen cells. Control cultures only received X-irradiated spleen cells. After 7 days each microwell was tested for cytotoxicity against transfected L cells expressing *MCMV* and H-2L^d.

Frequencies of Th cells were determined as described (34, 36). Briefly, LN T cells were removed 5 (KLH) or 7 days (*L. monocytogenes*) after immunization, and placed in microcultures together with 10^4 syngeneic B cells and 100 μg/ml NIP_{20}-KLH or 5×10^6 heat-killed *L. monocytogenes*. Seven days later, the specific IgM response was determined by ELISA (34, 36). In all LD experiments each cell concentration was set up in 24 parallel cultures.

T cell bulk cultures. Day 8 *MCMV*-immune LN T cells (3×10^6/ml) were expanded in 25-cm^2 cell culture flasks (Falcon, Becton Dickinson, Mountain View, CA) on syngeneic, nonimmune, irradiated spleen cells (3×10^6/ml) in 5 ml RPMI 1640 containing 10% ConASN for 7 days. Cytotoxicity of CTL was analyzed at indicated E/T cell ratios using lines L/L^d or L/ie 1-L^d/2 as targets. C3H LN T cells (3×10^6/ml) were cultured together with irradiated BALB/c spleen cells (3×10^6/ml) as described above. Cytotoxicity of allospecific CTL was analysed on lines L/L^d or Ltk$^-$ at indicated E/T cell ratios.

Assay for cytotoxicity. Cytotoxicity was measured in a standard 4-h ^{51}Cr-release assay as previously described (33). The T cell lymphoma RBL 5, derived from a C57BL/6 mouse (H-2^b) (37) was kindly donated by Dr. H.-U. Weltzien, Freiburg, FRG, and used with and without TNP modification as target cells for H-2^b-restricted TNP-specific cytotoxicity. Lines L/L^d and L/ie 1-L^d/2, Ltk$^-$, derived from C3H/An mice (H-2^k) and transfected with the H-2L^d or cotransfected with the H-2L^d and *MCMV* gene 1, respectively, were used as target cells for CTL (26).

Assay for proliferation. The number of cultures with growing cells was determined after 7 days by visual examination with an inverted microscope (31).

Frequency estimates. CTL-p frequencies were calculated using minimum χ^2 estimation. The mean ($\bar{X}$) plus 3 SD (σ) of cultures was used to determine the threshold to calculate the fraction of nonresponding cultures (31). According to Taswell (38) an estimation was accepted when the probability value was $p > 0.05$.

RESULTS

L/ie 1-L^d/2 target cells are only lysed by in vivo MCMV-primed CTL specific for IE 1 and restricted to L^d. In this study, L cells transfected with *MCMV*-ie 1 as well as H-2L^d are used as target cells for in vivo *MCMV*-primed CTL. *MCMV*-IE 1-specific, L^d-restricted CTL lyse L/ie 1-L^d/2 cells (26), but do not recognize L cells (endogenous haplotype H-2^k) expressing L^d only (39) (Fig. 1*A*). L cells transfected with L^d only, are lysed by CTL derived from a MLR (C3H anti-BALB/c; Fig. 1*B*). Similarly, addition of the peptide representing the epitope sequences of the ie 1 gene product renders L cells expressing L^d but not L cells expressing K^d or D^d susceptible to the attack by *MCMV*-IE 1-specific CTL (27). Thus, this target allows the selective analysis of IE 1-specific, H-2L^d-restricted CTL among *MCMV*-primed T cells.

Treatment of MCMV-primed T cells with anti-$V\beta_8$-antibody F23.1 and complement eliminates a major proportion of CTL recognizing the IE 1 (pp89) of MCMV together with H-2L^d. As a first attempt to analyze the contribution of $V\beta_8^+$ T cells to the CTL response against IE 1 and H-2L^d, T cells were twice treated with the antibody F23.1 and C to remove $V\beta_8^+$ cells. Aliquots of untreated (control) and $V\beta_8$-depleted T cells were analyzed by flow microfluorimetry to test their purity. Each T cell group was expanded in vitro in limiting dilution on syngeneic, X-irradiated, nonimmune spleen cells in the presence of ConASN as source of lymphokines. This expansion protocol (23) was chosen to avoid in vitro-selection by Ag. After 7 days, LD cultures were analyzed for their lytic potential on L/ie 1-L^d/2 cells. As a control normal unprimed T cells were included in the experiment. The results are shown in Figure 2*A*. As shown before (23),

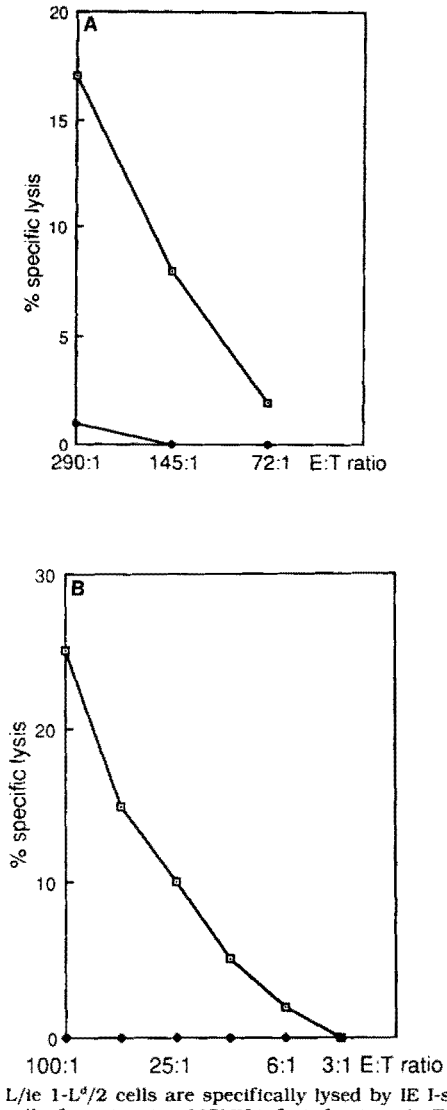

Figure 1. L/ie 1-L^d/2 cells are specifically lysed by IE I-specific, L^d-restricted T cells from in vivo *MCMV*-infected mice. *A*, T cells from infected mice lyse L/ie 1-L^d/2 target cells (□), but do not lyse L/L^d target cells (◆). *B*, L/L^d target cells (□) are lysed by H-2^d-specific CTL derived from a MLR (C3H anti-BALB/c). Nontransfected L cells are not lysed (◆). CTL were derived from bulk cultures as described in *Materials and Methods*.

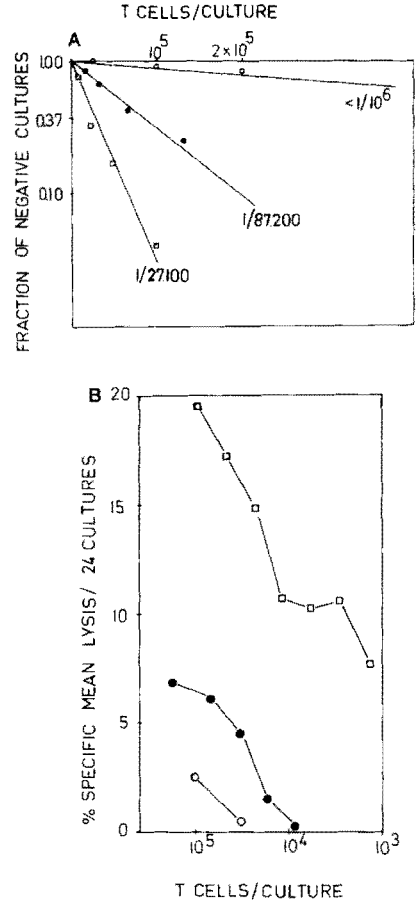

Figure 2. Depletion of $V\beta_8^+$ T cells reduces the frequency (*A*) and the lytic potential (*B*) of *MCMV*-IE 1-specific, H-2 L^d-restricted cytotoxic T cells. *A*, Normal LN T cells (○), *MCMV*-primed (day 8) LN T cells without (□) or after (●) treatment with F23.1 + C′ were cultured under LD conditions on syngeneic feeder cells in the presence of lymphokines and in the absence of viral Ag. After 8 days, each culture was tested for cytolytic activity on transfected targets expressing *MCMV*-ie 1 and H-2 L^d. Frequencies were determined using the minimal χ^2 method (35). All p values >0.05. The 95% confidence limits were 1/21.300 to 1/37.000 (□), and 1/67,000 −1/125.000 (●), respectively. *B*, LD cultures described in *A* were analyzed by calculating the mean specific lysis of each group as a function of the input cell concentration at the beginning of the cultures. *MCMV*-primed LN T cells without (□) and after (●) F23.1 + C′ treatment, normal LN T cells (○).

the CTL response entirely depends on the immunization in vivo (nonimmunized T cells: <1/10^6). Depletion of $V\beta_8^+$ T cells lead to a threefold decrease in the frequencies of CTL displaying the analyzed specificity (untreated primed T cells: 1/27.100, $V\beta_8$ depleted primed T cells: 1/87.200). This result is strengthened by comparing the lytic potential of the two subpopulations: In Figure 2*B* the mean lysis of 24 cultures is plotted vs the input cell concentration at the beginning of the culture period. The treatment of primed T cells with F23.1 and C reduced the lytic potential at least fivefold.

Cell sorter separation of T cells via F23.1 into $V\beta_8^+$ and $V\beta_8^-$ subpopulations. Nylon wool purified lymph node T cells (>95% Thy 1^+, <2.5% Ig^+) were separated into $V\beta_8^+$ and $V\beta_8^-$ T cell subpopulations. Separated T cells (>95% Thy 1^+) were reanalyzed to test their purity. An example of such separation and reanalysis is shown in Figure 3. In this experiment, positively selected T cells were 94% $V\beta_8^+$, negatively selected T cells 99.5% $V\beta_8^-$.

Preferential recognition of MCMV-IE 1 (pp89) and H-2L^d by $V\beta_8^+$ cytotoxic T cells. Frequencies of *MCMV*-IE 1-specific, L^d-restricted CTL-p were estimated in cell sorter separated $V\beta_8^+$ and $V\beta_8^-$ T cells of day 8 *MCMV*-immune mice. The results of two such experiments are shown in Table I. In the first experiment the relative CTL-p frequencies increased from 1/39.500 to 1/22.300 (comparing unseparated LN cells with $V\beta_8^+$ cells), or decreased to 1/62.700 (comparing unseparated LN cells to $V\beta_8^-$ cells), respectively. This result was clearly confirmed in a second experiment: total LN cells contained CTL-p recognizing the L/ie 1-L^d/2 targets in a frequency of 1/17.000 (purified T cells: 1/14.500, T cells treated with F23.1 and anti-mouse Ig^{FITC} conjugate: 1/22.700). Again, the frequencies of the positively and negatively selected T cell populations deviated significantly from these control frequencies. Only 1/57.000 $V\beta_8^-$ T cells recognized the *MCMV*-IE I together with H-2L^d, as opposed to 1/9.900 $V\beta_8^+$ T cells.

Exclusion of sorting procedure as source of frequency differences between F23.1$^+$ and F23.1$^-$ cell populations. In experiment II in Table I we have excluded that

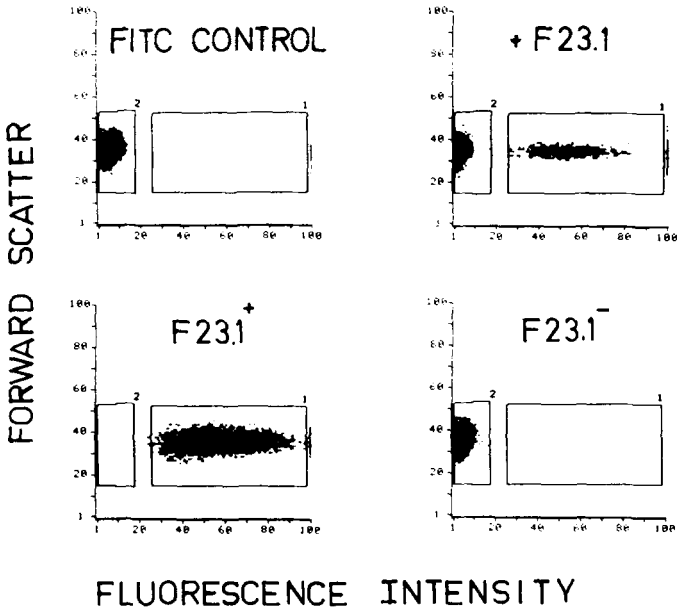

Figure 3. Separation of T cells into $V\beta_8^+$ and $V\beta_8^-$ subpopulations by flow microfluorimetry. For the FITC-control cells were stained with the anti Mlg^{FITC} only (region 1 contains 2.4% cells). To sort for and against $V\beta_8$ expression, T cells were stained with F23.1 and subsequently with the FITC conjugate (+F23.1: R 1 = 29%). Aliquots of the separated cells were reanalyzed to determine the purity of the separation at the end of the sorting process ($F23.1^+$: R 1 = 94%, and $F23.1^-$: R 1 = 0.5%). Staining was performed as described in *Materials and Methods*.

TABLE I

Frequencies of IE Ag 1-specific, H-2L^d-restricted CTL-p

Cell Population[a]	Expt. 1	Expt. 2
LN cells	1/39.500	1/17.000
T cells	ND	1/14.500
T + F23.1 + a Mlg^{FITC}[b]	ND	1/22.700
$F23.1^+$	1/22.300	1/9.900
	(1/17.000–1/32.400)[c]	(1/7.600–1/14.100)
$F23.1^-$	1/62.700	1/57.000
	(1/45.000–1/95.000)	(1/45.000–1/76.000)

[a] All analyzed cell populations were prepared from the same LN preparation. $F23.1^+$ and $F23.1^-$ subpopulations were separated by flow cytometry as described in *Materials and Methods*. Frequencies were estimated as described in the legend of Figure 3. All p values >0.05.

[b] Control T cells were stained with $F23.1^+$ conjugate but not separated on the sorter.

[c] The 95% confidence limits.

the staining of T cells with F23.1 and FITC-anti-Ig, binding to $F23.1^+$ but not $F23.1^-$ T cells, significantly alters the frequency estimates of unseparated T cells in limiting dilution cultures. It is possible, however, that an unknown factor introduced by the sorting procedure leads to a generally increased reactivity of $F23.1^+$ vs $F23.1^-$ T cells. To exclude this possibility frequency estimates of F23.1 and FITC-conjugate treated and sorted T cells were compared in other specific responses. Representative examples of these experiments are shown in Table II. Neither the frequencies of TNP-specific CTL-p nor of T cells proliferating in response to TNP-modified syngeneic spleen cells were influenced by treatment of unseparated T cells with the antibody F23.1 and FITC conjugate. Frequencies of alloreactive CTL-p (BALB/c, H-2^d anti-H-2^b), and of KLH- or *L. monocytogenes*-specific Th (in vivo immunized BALB/c or C57BL/6) are equally distributed between $V\beta_8^+$ and $V\beta_8^-$ populations.

DISCUSSION

We test the assumption that T cells expressing a restricted choice of Vβ-genes may possess a limited Ag-specific, MHC-restricted specificity repertoire. Unlike other investigators who obtained information on this question by the analysis of often relatively small numbers of T cell clones or T cell hybridomas (9–17), we determined frequencies of cell-sorter purified $V\beta_8^+$ and $V\beta_8^-$ polyclonal T cells after activation in vivo by a natural viral pathogen, the murine *CMV*. The use of H-2- and viral-Ag-transfectants as readout targets allowed the exact characterization of both Ag-specificity and MHC-restriction of CTL in this analysis.

$V\beta_8^+$ TCR were previously reported to be expressed on both MHC class I- and class II-restricted T cell subsets (6, 8) and to provide similar precursor frequencies for CTL with allospecificity (6), including anti-bm1 and anti-bm14 alloresponses (40) as $V\beta_8^-$ TCR. On the basis of a predominant $V\beta_8$ expression in a panel of I-E^d-restricted T cell clones with specificity for sperm whale myoglobulin peptides, Morel et al. (17) proposed a correlation of $V\beta_8$-expression and restriction to I-E^d. $V\beta_8^+$ T cells were recently reported to determine disease susceptibility in two models of autoimmunity: the response against the auto-Ag myelin basic protein causing autoimmune encephalitis (18, 19), and in collagen-induced arthritis (20). Together with our present results, the overall impression from these experiments is that immune responses to well-defined epitope/MHC combinations may reveal a restricted Vβ-gene-usage (17, 18), whereas responses to multiple epitope/MHC combinations are less likely to do so. Although this is not surprising, it is interesting to consider why the anti-bm14 responses appear to fall into the latter category. The apparent heterogeneity in the responses against bm-mutants (40) could be explained by the presentation of different sets of peptides by wildtype and mutant H-2 class I molecules.

Here we provide evidence for the preferential recognition by $V\beta_8^+$ cytotoxic T cells of a nonstructural viral Ag (*MCMV*-IE I) together with H-2L^d in BALB/c mice. T cells with specificity for *MCMV*-IE I were previously shown to constitute 50% of all CTL activated by *MCMV* in vivo (24). A large proportion of these CTL (≈25%) detect the epitope exposed on L/ie I-L^d/2 cells (26) which can minimally be defined as the pentapeptide sequence his-phe-met-pro-thr (30). The frequencies of CTL exhibiting this defined specificity and restriction are three- to fivefold greater in the $V\beta_8^+$ than in $V\beta_8^-$ T cell repertoire. In the local popliteal lymphnode frequencies of unseparated ($V\beta_8^+$ and $V\beta_8^-$) CTL anti-IE I + H-2L^d increased from less than 1/10^6 in nonimmunized mice to 1/27.000 in primed mice (37-fold), whereas the frequencies of $V\beta_8$-depleted CTL increased from 1/10^6 to 1/87.000 (12-fold) (Fig. 2*A*). It is not clear, whether the observed preferential recognition of pp89 + L^d by $V\beta_8^+$ CTL is due to the particular Ag which is presented in the context of L^d, or whether it is due to the H-2L^d itself, or due to this Ag/MHC combination. The reason for these frequency differences could be that T cell receptors with specificity for IE 1 and L^d are more frequent among the $V\beta_8^+$ than among the $V\beta_8^-$ T cell subpopulations. An alternative explanation could be that $V\beta_8^+$ cytotoxic T cells are expanding to greater clone sizes and are therefore more often detected in the limiting dilution assay. In this context it is interesting to note that after priming in vivo and sorting, only $F23.1^+$ T cells could be induced to proliferate in vitro in response to myelin basic protein peptides (19).

TABLE II

Frequencies of proliferating, cytotoxic and Th cells of various specificities analyzed in $V\beta_8^+$ and $V\beta_8^-$ T cell populations

Function and Specificity[b]	Cell Populations[a]				
	T	T + F23.1 + aMig^{FITC}	F23.1$^+$	F23.1$^-$	F[c]
1. TNP-specific ProTL-p	1/5.800	1/7.800	ND	ND	
2. TNP-specific CTL-p	1/18.500	1/15.000	ND	ND	
	1/16.500	1/17.800	1/15.900	1/25.800	1.6
3. Alloreactive CTL-p (H-2^d anti H-2^b)	1/1490	ND	1/2020	1/1970	0.9
4. KLH-specific Th	1/4310	1/2160	1/2640	1/2960	1.1
5. L. monocytogenes specific Th	1/4180	1/2725	1/4310	1/3100	0.7

[a] Compare Table I for details.
[b] LN T cells from normal (1–3) or immunized (4, 5) BALB/c (3, 4) or C57BL/6 (1, 2, 5) mice were analyzed in experiments as described in *Materials and Methods*.
[c] Frequency observed in F23.1$^+$/frequency observed in F23.1$^-$.

Our experiments were designed to avoid Ag-driven T cell expansion in vitro, i.e., T cells were cultured at the clonal level directly from *MCMV*-immune lymphnodes in the absence of viral Ag. We therefore propose that the nonstructural IE protein pp89 together with H-2L^d is preferentially recognized by $V\beta_8^+$ CTL in vivo. It would therefore be of interest to analyze whether the preferential recognition of *MCMV*-IE 1 by $V\beta_8^+$ T cells results in a greater resistance of $V\beta_8^+$ mouse strains than of $V\beta_8^-$ mouse strains to *MCMV* infection. However, $V\beta_8^-$ CTL are also capable to recognize IE I + L^d and hence we do not expect a total loss of recognition in $V\beta_8^-$ mouse strains. Moreover, T cell and B cell responses to other viral Ag could also contribute to the resistance against *MCMV* infection. Nevertheless, this could be a further example of TCR gene controlled immune responsiveness similar to those described elsewhere (18–20) and by Epstein et al. (41) who traced one of two immune-response genes in an anti H-Y CTL response to the TCR-α-chain locus.

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