Highly Protective In Vivo Function of Cytomegalovirus IE1 Epitope-Specific Memory CD8 T Cells Purified by T-Cell Receptor-Based Cell Sorting

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Reconstitution of antiviral CD8 T cells is essential for controlling cytomegalovirus (CMV) infection after bone marrow transplantation. Accordingly, polyclonal CD8 T cells derived from BALB/c mice infected with murine CMV protect immunocompromised adoptive transfer recipients against CMV disease. The protective population comprises CD8 T cells with T-cell receptors (TCRs) specific for defined and for as-yet-unknown viral epitopes, as well as a majority of nonprotective cells with unrelated specificities. Defined epitopes include IE1/m123 and m164, which are immunodominant in terms of the magnitude of the CD8 T-cell response, and a panel of subordinate epitopes (m04, m18, M45, M83, and M84). While cytolytic T-lymphocyte lines (CTLs) were shown to be protective regardless of the immunodominance of the respective epitope, the individual contributions of in vivo resident epitope-specific CD8 T cells to the antiviral control awaited investigation. The IE1 peptide 168-YPHFMPNTL-176 is generated from the immediate-early protein 1 (IE1) (pp89/76) of murine CMV and is presented by the major histocompatibility complex class I (MHC-I) molecule Ld. To quantitate its contribution to the protective potential of a CD8-T memory (CD8-TM) cell population, IE1-TCR+ and IE1-TCR− CD8-TM cells were purified by epitope-specific cell sorting with IE1 peptide-loaded MHC-immunoglobulin G1 dimers as ligands of cognate TCRs. Of relevance for clinical approaches to an adoptive cellular immunotherapy, sorted IE1 epitope-specific CD8-TM cells proved to be exceedingly protective upon adoptive transfer. Compared with CTLs specific for the same epitope and of comparable avidity and TCR β-chain variable region (Vβ)-defined polyclonality, sorted CD8-TM cells proved to be superior by more than 2 orders of magnitude.

The Ld-restricted immediate-early 1 (IE1) peptide 168-YPHFMPNTL-176 of murine cytomegalovirus (mCMV) was the first antigenic peptide to be identified for a herpesvirus (50). The IE1 protein derived from open reading frame (ORF) m123, an intranuclear phosphoprotein which exists in molecular species of 89 and 76 kDa (32), is expressed in the IE phase of viral gene expression and performs regulatory and transactivating functions (12, 31, 39). It is encoded in transcription unit ie1/3 of which mRNAs specifying proteins IE1 (encoded by exons 2, 3, and 4) and IE3 (encoded by exons 2, 3, and 5) are generated by differential splicing (31, 39). The IE1 protein is processed to yield peptide 168-YPHFMPNTL-176 and an N-terminally elongated precursor 166-DMYPHFMPNTL-176 by the constitutive proteasome and, more efficiently, by the immunoproteasome, with only the precursor being translocated into the lumen of the endoplasmic reticulum for major histocompatibility complex class I (MHC-I) loading (36). N-terminal trimming finally leads to the IE1 peptide presented by the MHC-I molecule Ld (reviewed in reference 45).

Based on the frequency of IE1 epitope-specific CD8 T cells primed during acute infection and on the establishment of long-term IE1-specific memory, the IE1 peptide was classified as one of just two immunodominant MHC-I-restricted antigenic peptides in the H-2d haplotype (25). Owing to MHC polymorphism, it is evident that immunodominance of peptides and the proteins from which they are derived cannot be extrapolated to other haplotypes in the same animal species. This is all the more true for extrapolation from mCMV to antigenic peptides of human cytomegalovirus (hCMV) presented by HLA molecules. Thus, although an early report by Borysiewicz et al. (4) had indicated the CD8 T-cell immunogenicity of the hCMV IE1 ortholog, the predictive value of the BALB/c mouse model in terms of the role of IE proteins in immunity to CMVs has been debated for a long time, until more recently the IE1 immunogenicity in humans was revisited with unbiased new methodology (10, 14, 33, 34). Most intriguingly, in a comprehensive pangenomic search for antigenic ORFs of hCMV by using overlapping peptides and by covering all major HLA molecules present in the human population, Louis Picker and colleagues identified ORF UL123 encoding the IE1 protein as one of the top three antigen-encoding ORFs of hCMV that are most frequently detected by HLA class I-restricted human CD8 T cells (L. Picker, “T cell recognition of hCMV in natural human infection: pan-genome analysis of immunogenic open reading frames,” Instituto Juan March de Estudios e Investigaciones workshop “Immunodominance: the key to understanding and manipulating CD8 T cell responses...
to viruses,” Madrid, Spain, 2004; L. Picker, personal communication). Thus, in this specific aspect of immunity to CMVs, the BALB/c mouse model matches the situation found in approximately half of all hCMV-infected individuals; from this viewpoint, the BALB/c mouse model gains increasing importance as an in vivo experimental model for analyzing the contribution of IE1-specific CD8 T cells to the control of CMV disease.

At first glance, one may argue that this issue is long settled, as several previous approaches, including immunization with vaccinia virus recombinants expressing the IE1 protein (29) or the isolated IE1 peptide (6), immunization with synthetic IE1 peptide (55), genetic immunization with IE1-expressing plasmids (11), and adoptive transfer of IE1 epitope-specific cytolytic T-cell lines (CTLs) (24, 25), have bona fide demonstrated an in vivo protective antiviral effector function of IE1-specific CD8 T cells. Since polyclonal CD8 T-cell populations isolated from BALB/c mice during acute or latent infection from lymphoid organs (47, 49, 52) or from infiltrates at tissue sites of CMV disease (43) were found to be protective too, it was somehow logical to take it for granted that IE1-specific CD8 T cells are a major component of the in vivo CD8 T-cell response that resolves productive infection and prevents multiple-organ CMV disease (44). However, in a formal scientific sense, such an extrapolation is no proof. So, one important link was actually missing in the chain of evidence, namely, the direct demonstration of a protective antiviral function of ex vivo-isolated IE1-specific CD8 T cells.

Here, we have combined T cell receptor (TCR)-based epitope-specific cytotoxic flow cytometric cell sorting and adoptive immunotherapy of CMV infection to compare the protective function of IE1-TCR+ CD8 T cells with that of the remaining IE1-TCR− population comprising CD8 T cells specific for m164, the second immunodominant epitope (25), and CD8 T cells specific for the panel of known subordinate epitopes m04, m18, M45, M83, and M84 (45). The data show for the first time that ex vivo isolated IE1 epitope-specific memory CD8 T cells (CD8-TM cells) are highly protective.

MATERIALS AND METHODS

Infection and adoptive cell transfer. For immunological priming of CD8 T-cell donors, 8- to 11-week-old, immunocompetent, female BALB/c mice (H-2d haplotype) were infected in the left hind footpad (intraplantar infection) with 10^7 PFU of cell culture-propagated, sucrose gradient-purified (42) wild-type mCMV (mCMV-WT), strain Smith ATCC VR-194, recently reaccessioned as VR-1399. PFU of cell culture-propagated, sucrose gradient-purified (42), wild-type mCMV (mCMV-WT) were as follows: IE1/Ld, 168-YPHFMPTNL-176 (50); m04/Dd, 243-YG (46); m164/Dd, 209-YG (46); m18/Dd, 203-YG (46); m164/Ld, 243-YG (46). The amount of peptide-loaded donors required for an optimal stimulation of cells depends on the frequency of cells that express a cognate TCR in a cell population as well as on the number of TCR molecules per cell. Titration of IE1 dimers revealed an optimal staining at 5 μg per 10^6 cells in the case of a monospecific IE1-CTLL and 1 μg per 10^6 cells in the case of ex vivo isolated CD8-TM cells from the spleen. For specificity control of the staining, Ld-Dimer-NP9/CMV was used at a concentration of 10 μg per 10^6 cell in all cases. It should be noted that dimers not loaded with exogenous peptide can accommodate multiple endogenous peptides, which led to a somewhat higher background staining in polyspecific CD8 T-cell populations (data not shown).

Effector cells. Memory cells were pooled from spleens of at least three BALB/c mice at ≥3 months after infection, time points at which productive primary infection was resolved. They were either used for analyses (see below) or served as a source for the generation of IE1-CTLs.

(i) Immunomagnetic cell sorting. For subsequent cytotoxic flow cytometric analyses (with exceptions specified), for intracellular cytokine staining, and for cell sorting, CD8 T cells were enriched by one round of positive immunomagnetic cell sorting with the autoMACS system (Miltenyi Biotec Systems, Bergisch-Gladbach, Germany) following the protocol recommended by the manufacturer. For enzymylated immunomospot (ELISPOT) assays (see below), two parallel runs of automated magnetic cell sorting (autoMACS sorting) were performed to reach >95% purity. In brief, up to 10^7 cells were resuspended in 90 μl of running buffer (2 mM EDTA in phosphate-buffered saline containing 0.5% [wt/vol] bovine serum albumin) and mixed with 10 μl of rat anti-mouse CD8α (Ly-2) MicroBeads (Miltenyi catalog no. 130-049-011). After 15 min of incubation at 4°C in the dark, followed by washing and resuspension in running buffer, immunomagnetic sorting was performed by using Posset and Possel skirt separation programs (Miltenyi) for one- and two-column separation, respectively.

(ii) Cytotoxic flow cytometric cell sorting. For the purification of IE1-specific CD8-TM (IE1-TCR+ CD8+), immunomagnetically preenriched CD8 T cells (see above) were saturated with normal goat serum (Jackson ImmunoResearch Laboratories catalog no. 005-000-121) and rat monoclonal antibody (MAB) anti-mouse CD16/ CD32 (anti Fcy III/II receptor, clone 2.4G2; BD Biosciences Pharmingen catalog no. 531142), and were labeled with fluorescent (isothiocyanate; FITC)-conjugated rat MAB anti-mouse CD8α (clone 53-6.7; BD Biosciences Pharmingen catalog no. 553031) and IE1-IP peptide-loaded dimer XI H-2Ld/Ig (see above) with PE-conjugated rat MAB anti-mouse IgG1 (clone A45-1; BD Biosciences Pharmingen catalog no. 550803) as a second antibody. The cell sorter ingenus was an EPICS Altra HyperSort (Beckman Coulter, Fullerton, Calif.) equipped with a 100-μm nozzle and operated with EXP302 acquisition software, version 2.0. The laser wavelength was 488 nm. Band-pass filters for 525 and 575 nm were used to measure FITC and PE fluorescence, respectively. Cytomix RXP Analysis software, version 1.0, was used for data processing. Sort gates were set on living cells with positive FL-1 (FITC) and high FL-2 (PE) fluorescence, discarding CD8 T cells with low expression of the IE1-TCR. Sorting was performed in

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AltralSort mode 3, with a flow rate of ca. 5,000 cells/s. Recovered cells were collected in fetal calf serum-saturated polyethylene tubes.

(iii) Generation of epitope-specific CTLLs and cytolytic assay. IE1-CTLLs were generated by repeated restimulation of memory cells with synthetic IE1 peptide (25). In brief, 1.5 × 10^5 unseparated memory spleen cells were seeded per 2-ml well (24-well flat-bottom culture plates) in 1.5 ml of medium supplemented with synthetic IE1 peptide in a concentration of 10^-4 M. At day 4, 100 U of recombinant human interleukin-2 (IL-2) (H-2b) was added to 0.5 ml of fresh medium from the next restimulation was performed on day 7 by a 1:2 split of the cultures and a supply of 1 ml of fresh medium supplemented with 10^-4 M of IE1 peptide, 200 U of recombinant human IL-2, and 8 × 10^5 γ-irradiated (90 Gy) P815-B7 cells (2) as stimulator cells. On day 14 and every second week thereafter, further rounds of restimulation were performed after the number of effector cells was adjusted to 5 × 10^5 per well. In the intervals between the restimulations, cultures were split and refed with the IL-2 medium when required. The experiments described here were performed with IE1-CTLLs propagated for three (adoptive transfer experiments) to not more than six (in vitro experiments) rounds of restimulation and ~1 week after the last restimulation, so that stimulator cells were largely absent at the time of harvest. Cytolytic activity was measured by a standard 31Cr release assay with the D8A-2/2 mouse (H-2a)-derived P815 mastoma targets, targets that were pulsed with 10^-3 M IE1 peptide for measuring IE1 epitope-specific cytolytic activity or were left without exogenous peptide for measuring noncognate lysis of the target cells.

Two-color cytofluorometric analysis of epitope-specific CD8 T cells. Cells of an IE1-CTL or immunomagnetically enriched CD8 T cells were blocked against Fc receptor binding (as in the cell sorting protocol above) and were labeled with peptide-loaded dimers or peptide-folded PE-conjugated tetramers (see above) for 30 min at 4°C. In the case of the nonfluorochromated dimers, cells were washed, resuspended in 50 μl of cytometry buffer, and stained for 15 min with PE-conjugated rat anti-mouse MAb anti-mouse IgG1 (see above). In both cases, after a washing step, cells were finally labeled with FITC-conjugated rat anti-mouse anti-mouse CD8α (see above). All labeling procedures were performed on ice to minimize T-cell activation, receptor capping, and receptor internalization. The analysis was performed with a FACS sorter (Becton Dickinson) using CellQuest 3.3 software for data processing. For quantification of an epitope-specific CTLL was unknown.

RESULTS

Selection of an IE1 epitope-specific polyclonal CTL. The principal question of whether IE1 epitope-specific CD8 T cells exert a protective antiviral function in vivo has already been indirectly answered in the positive by adoptive cell transfer of IE1-CTLs (24, 25). However, since for technical reasons ex vivo IE1 epitope-specific CD8 T cells generated during a natural, polyclonal, and polyspecific immune response to infection were not previously accessible to direct testing, in vivo antiviral efficacies could not be compared. Consequently, the influence of in vitro selection on the antiviral function during the generation of an epitope-specific CTL was unknown.

Here, we have undertaken every effort to keep the in vitro selection to an unavoidable minimum to generate a CTL that is as close to its ex vivo precursors as possible. The criterion for the selection that defines the minimal, i.e., necessary, number of in vitro restimulations was an effector cell population in which all cells expressed an L^4, IE1 peptide-specific TCR, designated IE1-TCR. Criteria for the selection that defines the maximal, i.e., still tolerable, number of in vitro restimulations were the maintenance of polyclonality and maintained expression of the coreceptor molecule CD8.
The TCR β-chain variable region (Vβ) expression pattern was used to evaluate gross shifts in clonal composition during CTLL selection. To get comparative figures, we first recorded the Vβ expression patterns for ex vivo-isolated CD8 T-cell populations of BALB/c mice (Fig. 1). At a glance, the patterns were very similar for CD8 T cells present in the spleens of adult mice naive in terms of immunity to mCMV, in draining popliteal lymph nodes at the peak of an acute immune response on day 8 after intraplantar mCMV infection, and during early memory in the spleens of mice 10 weeks after intraplantar primary infection (Fig. 1A). Thus, apparently, mCMV infection had no significant impact on overall Vβ usage in the CD8 T-cell pool of BALB/c mice. Since IE1 epitope-specific CD8-TM cells are only a minority constituent of primed CD8 T-cell pools, we next directly compared Vβ usage by all CD8 T cells and by IE1-specific CD8 T cells derived from spleens in a late memory phase 28 weeks after primary infection (Fig. 1B). Early and late memory Vβ usage patterns were almost identical. Importantly, the pattern for IE1 epitope-specific CD8 T cells largely mirrored the pattern observed with all CD8 T cells.

The late memory spleen cell population was the one used as the source for the selection of IE1-CTLL (Fig. 2), and the Vβ expression pattern among all T cells was monitored during six rounds of in vitro restimulation with IE1 peptide (Fig. 2A). In essence, the Vβ expression pattern was quite similar to the ex vivo pattern of the starting population, with Vβ8.1-Vβ8.2 predominating throughout. Importantly, the pattern was fairly robust in the course of the restimulations. Apart from minor, barely significant fluctuation, the important information is that the IE1-CTLLs remained polyclonal during the observation period and differed little from the starting population. It must be emphasized that this relative stability was a feature repeatedly observed for IE1-specific CTLLs, whereas CTLLs of other specificities tended to show more rapid Vβ selection, though the expanded Vβ family cells can vary between individual CTLLs of the same epitope specificity (data not shown).

The polyclonality revealed by the Vβ expression pattern of the IE1-CTLL does not reflect absence of epitope-specific selection. As shown in Fig. 2B, the proportion of IE1-specific cells among the CD8 T cells increased rapidly, and epitope monospecificity of the still-polyclonal CTLL was reached with the third restimulation and remained stable thereafter. Thus, three restimulations were necessary and sufficient for the generation of a monospecific IE1-CTLL. Combined, these findings show that many different TCRs recognize the L^d-IE1 peptide complex.

Since the avidity of binding to an MHC-peptide complex is known to be enhanced by the coreceptor molecule CD8, expression of CD8 on a CTLL may have an impact on its antiviral function. As shown in Fig. 2C, CD4 T cells were lost from the starting spleen cell population, and a pure IE1-TCR^+ CD8^+ cell line was generated after the third restimulation. From then on, however, CD8 expression was gradually lost, yielding a subpopulation within the IE1-CTLL that displayed the phenotype IE1-TCR^+ CD8^-.

According to the selection criteria defined above, three restimulations were defined for studies of in vivo antiviral efficacy as the optimal condition for the generation of a polyclonal
Frequency of IE1-specific memory CD8 T cells: comparison between tetramer and dimer staining. It is a problem inherent in TCR-based epitope-specific cell sorting that cross-linking of TCR molecules by multivalent MHC-peptide reagents leads to signal transduction that can activate effector T cells to instant delivery of effector functions and subsequent apoptosis or can induce anergy in resting T cells. This can potentially counteract the use of the sorted cells in functional assays in vitro, as well as in vivo (1, 35). On the other hand, the affinity of peptide-loaded MHC-I monomers is too low for stable binding to the TCR (3, 5, 38). In accordance with the literature on TCR binding affinities of MHC multimers (for a review, see reference 61), we reasoned that a dimeric tool, such as peptide-loaded MHC-IgG1 hybrids in which each of the two IgG1 heavy chains carries an MHC-I α-chain covalently linked to its V region (27, 56), binds less tightly and causes less cross-linking than peptide-folded MHC-I tetramers or higher-order multimers. Therefore, the use of dimers may be a compromise.

With this rationale in mind and for the specific case of the high-affinity Ld-restricted IE1 peptide, we first compared LdTetra-IE1mCMV and LdDimer-IE1mCMV for staining intensity and for the frequencies of detected IE1-TCR CD8 T cells in a polyclonal but monospecific IE1-CTLL (Fig. 3A), as well as in a polyclonal CD8-TM population derived from the spleens of BALB/c mice during latent mCMV infection (Fig. 3B). For specificity control, we used LdTetra-NP118-126mCMV and LdDimer-NP118-126mCMV that bind only to unrelated TCRs specific for the Ld-restricted NP118-126 epitope of LCMV. At a glance, the dimer was as good as the tetramer in terms of detected frequencies of IE1-TCR CD8 T cells and staining (here, PE/FL-2 fluorescence) intensity. In both instances, staining was highly specific.

Effect of dimer and tetramer binding on TCR signaling indicated by the expression of IFN-γ. Intracellular cytokine staining was used to monitor the activation of CD8 T cells by ligation of their TCRs with dimers or tetramers. CD8 T cells of the IE1-CTLL, purified by one round of positive MACS sorting, were activated to express IFN-γ regardless of whether TCR ligation occurred with LdTetra-IE1mCMV or LdDimer-IE1mCMV (Fig. 4A). The level of activation was comparable to that observed after ligation with the physiological cell-bound ligand Ld-IE1mCMV provided by inter-CTL presentation of IE1 peptide added externally at an optimal concentration of 10^-8 M. Notably, CD8 molecule cross-linking in the process of MACS sorting already caused an elevated basal level of IFN-γ expression in the absence of TCR ligation. This was not a constitutive IFN-γ expression in CTLL, as it was not found in control experiments in which no MACS sorting was performed.
IE1mCMV and Ld Tetra-IE1mCMV differ in their influence on undialyzed peptide-loaded dimers (not shown). In the experiments in earlier experiments that had been performed with and lead to epitope-specific recognition. This was indeed an issue in earlier experiments that had been performed with PE-Ld Tetra-IE1mCMV. Control staining was performed with PE-Ld Tetra-NPmCMV. Tetramer, labeling of the IE1-TCR with PE-Ld Tetra-IE1mCMV. Control staining was performed with PE-Ld Tetra-NPmCMV. Displayed are dot plots of two-color cytofluorometric analyses with CD8 expression shown by FITC fluorescence (FL-1) intensity on the abscissa and IE1-TCR expression, as well as specificity control shown by PE fluorescence (FL-2) intensity, on the ordinate. Electronic gates were set on living lymphocytes and on positive FL-1 values to restrict the analyses to cells expressing CD8. The percentages of cells double positive for FL-1 and FL-2 are indicated in the upper-right quadrants. Dots represent ~4,000 IE1-CTL and ~8,000 CD8 T cells, respectively.

In conclusion, as predicted, TCR ligation activated the cells to express IFN-γ. However, these experiments did not reveal a significant difference between dimers and tetrarmers in this respect. Since the difference in binding affinities between dimeric and higher-order multimeric ligands is not debatable (61), the data likely indicate that both reagents triggered the maximum possible level of signaling. While these results reaffirmed a potential problem of TCR-based cell sorting, they did not give preference to either of the TCR binding reagents.

**Effect of dimer and tetramer binding on the cytolytic activity of IE1-specific CTLs.** We next investigated if LdDimer-IE1mCMV and Ld Tetra-IE1mCMV differ in their influence on the in vitro cytolytic activity of an IE1-CTL (Fig. 5). Notably, and somewhat unexpectedly in view of the literature (35), neither of these TCR binding and cross-linking reagents inhibited the recognition of target cells that presented the IE1 peptide. This finding implies that a sufficient amount of free IE1 peptide was available for recognition of the Ld-IE1 peptide complexes on the target cells and that CTL apoptosis was not a critical factor during the 4-h assay period. Though most of the target cell lysis was specific in that it required presentation of the IE1 peptide, some lysis of target cells in absence of added IE1 peptide occurred after TCR-mediated signal transduction elicited by either of the two reagents. Yet, one has to consider that free residual peptide present in the reagents from the dimer-loading or tetramer-folding process and, alternatively or in addition, peptide released from the peptide-MHC complexes can bind to the class I molecules on the target cells and lead to epitope-specific recognition. This was indeed an issue in earlier experiments that had been performed with undialyzed peptide-loaded dimers (not shown). In the experiment with results shown in Fig. 5, however, the supernatant of IE1-CTLs that were stained with dialyzed IE1 peptide-loaded dimers did not contain a sufficient concentration of free IE1 peptide (>10^{-12} M) to render peptide-unpulsed target cells susceptible to lysis by “untouched” IE1-CTL (data not shown). A second argument considered by us was the possibility that the IgG1 Fc-portions of the TCR-bound MHC-IgG1 hybrids may bind to Fc receptors on the peptide-unpulsed target cells and thereby provide a surrogate MHC-peptide complex, bridging the target cells with the CTL. However, extensive blocking of Fc receptors on the target cells did not inhibit the lysis (results not shown). We therefore concluded that activation of the CTL by TCR-cross-linking leads to some noncognate lytic activity, possibly by “bystander effects” such as release of perforin and granzymes, due to the activation by TCR cross-linking. Regardless, this phenomenon is not a specific problem of the use of dimers, as it occurred in like manner with tetrarmers (Fig. 5 and reference 35).

**Dimer binding to the IE1-TCR does not interfere with antiviral in vivo function.** According to the literature, binding of tetrarmers to TCRs can inhibit effector cell function (35). This limitation of tetramer technology in the preparative area of application originally gave the impetus for the development of the MHC-streptagII multimer system: briefly, streptamers that may bind to Fc receptors on the peptide-unpulsed target cells and thereby provide a surrogate MHC-peptide complex, bridging the target cells with the CTL. However, extensive blocking of Fc receptors on the target cells did not inhibit the lysis (results not shown). We therefore concluded that activation of the CTL by TCR-cross-linking leads to some noncognate lytic activity, possibly by “bystander effects” such as release of perforin and granzymes, due to the activation by TCR cross-linking. Regardless, this phenomenon is not a specific problem of the use of dimers, as it occurred in like manner with tetrarmers (Fig. 5 and reference 35).

**FIG. 3.** Frequencies of IE1 epitope-specific CD8 T cells. (A) Frequency in an IE1-CTL. (B) Frequency in a polyspecific population of CD8 T cells derived from spleens of three latently mCMV-infected mice at 4 months after infection. Dimer, labeling of the IE1-TCR with LdDimer-IE1mCMV and PE-conjugated second antibody directed against IgG1. Control staining was performed with LdDimer-NPmCMV. Tetramer, labeling of the IE1-TCR with PE-Ld Tetra-IE1mCMV. Control staining was performed with PE-Ld Tetra-NPmCMV. Displayed are dot plots of two-color cytofluorometric analyses with CD8 expression shown by FITC fluorescence (FL-1) intensity on the abscissa and IE1-TCR expression, as well as specificity control shown by PE fluorescence (FL-2) intensity, on the ordinate. Electronic gates were set on living lymphocytes and on positive FL-1 values to restrict the analyses to cells expressing CD8. The percentages of cells double positive for FL-1 and FL-2 are indicated in the upper-right quadrants. Dots represent ~4,000 IE1-CTL and ~8,000 CD8 T cells, respectively.

(data not shown). A corresponding experiment was done for MACS-purified CD8-T M cells (Fig. 4B) with similar results, except that the memory cells did not respond to CD8 molecule cross-linking.

In conclusion, as predicted, TCR ligation activated the cells to express IFN-γ. However, these experiments did not reveal a significant difference between dimers and tetrarmers in this respect. Since the difference in binding affinities between dimeric and higher-order multimeric ligands is not debatable (61), the data likely indicate that both reagents triggered the maximum possible level of signaling. While these results reaffirmed a potential problem of TCR-based cell sorting, they did not give preference to either of the TCR binding reagents.

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**FIG. 3.** Frequencies of IE1 epitope-specific CD8 T cells. (A) Frequency in an IE1-CTL. (B) Frequency in a polyspecific population of CD8 T cells derived from spleens of three latently mCMV-infected mice at 4 months after infection. Dimer, labeling of the IE1-TCR with LdDimer-IE1mCMV and PE-conjugated second antibody directed against IgG1. Control staining was performed with LdDimer-NPmCMV. Tetramer, labeling of the IE1-TCR with PE-Ld Tetra-IE1mCMV. Control staining was performed with PE-Ld Tetra-NPmCMV. Displayed are dot plots of two-color cytofluorometric analyses with CD8 expression shown by FITC fluorescence (FL-1) intensity on the abscissa and IE1-TCR expression, as well as specificity control shown by PE fluorescence (FL-2) intensity, on the ordinate. Electronic gates were set on living lymphocytes and on positive FL-1 values to restrict the analyses to cells expressing CD8. The percentages of cells double positive for FL-1 and FL-2 are indicated in the upper-right quadrants. Dots represent ~4,000 IE1-CTL and ~8,000 CD8 T cells, respectively.
We previously established a murine model of CD8 T-cell-based preemptive cytoimmunotherapy of CMV dissemination and multiple-organ CMV disease in the immunocompromised (total-body γ-irradiated) host after local subcutaneous infection (45, 52). The in vivo protective antiviral activity of primed polyclonal CD8 T cells or in vitro-selected epitope-specific CTLLs is usually determined between days 11 to 13 after infection and intravenous cell transfer. In this model, infected cells become detectable in tissue sections of key target organs, such as liver and lungs, at around day 6. Thus, transferred cells have time to recover in the host and will still come in time to control organ infection. As shown in Fig. 6 for IE1-CTL labeled with Ld-Dimer-IE1mCMV, stained IE1-TCRs are rapidly downmodulated and disappear from the cell surface after 24 h of in vitro culture. If this can be extrapolated to in vivo conditions, the bound reagent may be less of a problem than was previously surmised.

In fact, in a pilot experiment (Fig. 7A) involving the adoptive transfer of a constant number of $5 \times 10^5$ cells of a polyclonal IE1-CTL, the control of infection was $\sim 2 \log_{10}$ PFU in the lung and $\sim 4 \log_{10}$ PFU in the spleen, regardless of whether the IE1-CTL were left untouched (group I), labeled with Ld-Dimer-IE1mCMV (group II), or incubated with the epitope-unrelated reagent Ld-Dimer-NPLmCMV (group III). In a separate experiment performed with the third-restimulation IE1-CTLL (Fig. 2), graded cell numbers either left untouched or labeled with Ld-Dimer-IE1mCMV were transferred to reveal any differences in the dose-response curves (Fig. 7B). Yet, there was no difference observed in the lung and no significant difference in the spleen, which implies that none of the above-discussed...
problems of epitope-specific TCR staining are critical in this particular experimental model.

In conclusion, binding of \( \text{L}^\delta\text{Dimer-IE1}^{\text{mCMV}} \) to the IE1-TCRs in an IE1-CTL did not significantly interfere with protective antiviral in vivo function upon adoptive cell transfer.

**Ex vivo sort-purified IE1 epitope-specific CD8 T cells are highly protective.** The promising protection data obtained with IE1-CTLs led to a control of infection of \(<1 \log_{10} \text{PFU} \) in the lung and of \(<2 \log_{10} \text{PFU} \) in the spleen. Based on this published and here reproduced experience with CTL dose-response curves in the adoptive transfer model, we did not expect much from this pilot experiment and were therefore even more surprised to find a very impressive control of infection of almost \(3 \log_{10} \text{PFU} \) in the lung and \(~5 \log_{10} \text{PFU} \) in the spleen (Fig. 8, left). Moreover, these cells limited the infection in liver and adrenal glands by \(~3 \log_{10} \) to below the technical limit of IHC detection of infected cells in tissue sections (Fig. 8, right). The protective impact of this antiviral control in terms of prevention of viral histopathology is illustrated for the same experiment by the corresponding IHC images in Fig. 9. In the “no transfer” control group, extended areas of infection can be seen in whole-organ sections of the adrenal-suprarenal glands (Fig. 9A), and the liver is literally riddled with plaque-like lesions (Figs. 9B1 and B2). By contrast, transfer of \(10^4 \) sorted IE1 epitope-specific CD8 T cells completely prevented viral histopathology in these two organs (Fig. 9C and D).

**Superior antiviral activity of IE1 epitope-specific ex vivo CD8-TM cells is not explained by higher TCR affinity or avidity.** The extraordinary antiviral in vivo activity of sorted IE1-specific memory cells compared to polyclonal CTLL specific for the same epitope prompted investigation into the reason. An immediate idea was that propagation in vitro causes a loss of antigen binding avidity. Since clonotypes with high-affinity TCRs are likely to better withstand limited in vitro antigen presentation and to utilize IL-2 more efficiently, we considered it unlikely that in vitro selection favors the expansion of low-affinity clonotypes; rather, the opposite makes sense. However, downmodulation of accessory and coreceptor molecules of the immunological synapse (9) may weaken the target cell recog-

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**FIG. 7.** Antiviral in vivo activity of IE1-CTL labeled with TCR binding dimers. (A) Cells of a third-restimulation IE1-CTL were either left untouched (group I, open circles), labeled with \( L^\delta\text{Dimer-IE1}^{\text{mCMV}} \) (group II, closed circles), or incubated for control with the unrelated nonbinding reagent \( L^\delta\text{Dimer-IE1}^{\text{mCMV}} \) (group III, closed squares). A constant number of \(~5 \times 10^6 \) IE1-CTLs were adoptedively transferred to BALB/c indicator recipients that were immunocompromised by 6 Gy of total-body \( \gamma \) irradiation and were infected subcutaneously with \(~10^6 \) PFU of mCMV. Group \( \square \) (open squares), no cell transfer. Virus titers were determined in organ homogenates of lungs and spleen on day 12 after cell transfer. Symbols represent data for individual recipients. The median values are marked. (B) Transfer of the indicated graded cell numbers of another third-restimulation IE1-CTL (Fig. 2) that were either left untouched (open circles) or labeled with \( L^\delta\text{Dimer-IE1}^{\text{mCMV}} \) (closed circles).

**FIG. 8.** Antiviral in vivo activity of sort-purified IE1 epitope-specific CD8-T cells. Spleen cells were isolated from 5 BALB/c mice 3 months after acute mCMV infection. IE1-specific CD8 T cells were purified by cytofluorometric cell sorting (see the legend to Fig. 12 for a detailed sort protocol) using \( L^\delta\text{Dimer-IE1}^{\text{mCMV}} \) for epitope-specific TCR labeling. The in vivo-protective antiviral activity was tested by adoptive transfer of \(~10^4 \) sorted cells per immunocompromised recipient (closed circles). On day 12 after cell transfer, virus titers were determined for lungs and spleen (left), and the numbers of infected cells were determined for liver and adrenal (suprarenal) glands (right) in representative \(10 \) mm\(^2\) - by 2-\( \mu \)m tissue sections (see Fig. 9). Symbols represent data for individual transfer recipients. Median values are marked. Group \( \square \) (open squares), no cell transfer.
To cover both arguments, we determined by ELISPOT assay the minimal concentration of IE1 peptide that was needed to trigger TCR signaling in IE1 epitope-specific polyclonal CD8-TM ex vivo, as well as in a polyclonal third-restimulation IE1-CTLL derived thereof (Fig. 10). All IE1-specific cells in both populations were triggered to secrete IFN-γ by IE1 peptide concentrations of down to $10^{-9}\text{M}$. At $10^{-10}\text{M}$, ~50% of the IE1-specific CD8-TM cells failed to respond, while all cells of the IE1-CTLL were triggered. At the limit concentration of $10^{-12}\text{M}$, all IE1-specific CD8-TM cells failed, whereas the IE1-CTLL contained a detectable number of cells that were still capable of responding. In conclusion, as the overall TCR affinity-avidity was higher in the IE1-CTLL, this evidently is not the parameter accountable for its lower antiviral in vivo activity.

**Contribution of IE1 epitope-specific CD8 T cells to the protective capacity of a polyspecific CD8-TM population.** Referring to the immune response to mCMV in BALB/c mice, two immunodominant antigenic peptides (IE1 and m164) and several subordinate antigenic peptides (m04, m18, M45, M83, and M84) have been identified (for a review, see reference 45). While the two immunodominant peptides account for most of the mCMV-specific CD8 T-cell memory in latently infected mice (25) so that we do not expect the existence of an unidentified third immunodominant peptide, the list of subordinate peptides is likely to be incomplete. In fact, DNA vaccination experiments have recently predicted the existence of further
epitopes in protein M84 (63). Of course, the majority of CD8 T cells express TCRs that are unrelated in specificity to the priming viral antigens. The composition of the CD8 T-cell population in the group of mice used for a second sorting experiment was tested by an ELISPOT assay (Fig. 11). IE1-specific, IFN-γ-secreting CD8 T cells accounted for ~1% of the population, and the frequencies of CD8 T cells specific for the remaining epitopes added up to another ~1%. To separate IE1-specific and nonIE1-specific CD8 T cells, sort windows were set on IE1-TCR_{high} CD8^+ T cells as well as on IE1-TCR^− CD8^+ T cells (Fig. 12). Reanalysis documented an enrichment from 1.2 to >95% and from 85.5 to >99%, respectively. Finally, the protective antiviral capacity of the two sorted populations was compared by adoptive transfer of graded cell numbers into immunocompromised recipients infected with mCMV-WT (Fig. 13). The data fully confirmed the extraordinary antiviral activity of ex vivo-sorted IE1-specific CD8 T cells indicated by the first sorting experiment (Fig. 8 and 9). Remarkably, 400 cells were sufficient to control infection by ~1 log_{10} PFU in the lungs and ~3 log_{10} PFU in the spleen. Likewise, the number of infected cells was reduced by >2 log_{10} and ~1 log_{10} in liver and adrenal glands, respectively. In accordance with the presence of CD8-T_{M} cells specific for other protective mCMV epitopes, the IE1-TCR^− subset too was capable of controlling the infection. The seemingly lower efficacy per cell is explained by the fact that the majority of cells in this subset are specific for antigens unrelated to mCMV.

In conclusion, ex vivo sort-purified epitope-specific CD8-T_{M} are exceedingly protective in controlling CMV disease.

**DISCUSSION**

A role for IE antigen-specific CD8 T cells in the control of acute and latent CMV infections was originally proposed by Reddehase and Koszinowski on the occasion of the first description of mCMV IE protein immunodominance in the BALB/c mouse model of CMV infection (48). Since then, many lines of evidence in this model have indicated a protective antiviral function of mCMV IE1 (168-YPHFMPTNL-176) epitope-specific CD8 T cells in acute infection. Furthermore, the accumulation of activated CD62L^− IE1-specific effector-memory CD8 T cells (CD8-T_{EM}) in latently infected lungs (21) in which IE1 transcripts are sporadically generated (13, 37) also suggested a guardian function in the maintenance of viral latency (21, 43). Up to now, however, direct evidence for an antiviral function of host-resident IE1-specific CD8 T cells was missing, not least because technologies for a TCR-based ex vivo isolation of epitope-specific T cells were established only recently (35). Here, we used IE1 peptide-loaded MHC-IgG dimers for cytofluorometric sort-purification of ex vivo-derived CD8-T_{M} cells that express a cognate TCR. Adoptive cell transfer of these cells into immunocompromised indicator recipients demonstrated a highly efficient control of mCMV infection and prevention of viral histopathology. This completes the chain of evidence to conclude that IE1 epitope-specific CD8 T cells play a significant role in the control of CMV disease in the BALB/c mouse model.

Although no animal model can fully match clinical CMV in all its aspects, and even laboratory strains and selected clinical isolates of hCMV will not do so for all human individuals, the BALB/c mouse model is revalued by the increasing evidence that the IE1 protein of hCMV is a major source of CD8 T-cell epitopes for HLA molecules represented in a large proportion of the human population (see the introduction and reference 45 for an overview). However, as shown recently for the example of an immunodominant DP^b-restricted epitope in mCMV protein M45, the magnitude of an epitope-specific CD8 T-cell response does not always predict a role in antiviral protection (23). So, defining IE1 epitopes and IE1 epitope-specific CD8 T-cell frequencies in humans is no substitute for functional evidence of an antiviral protective activity. To the best of our knowledge, there exists only one report addressing the question of a contribution of IE1-specific CD8 T cells to protection against human CMV disease. Specifically, as reported by Hebart et al. (15) for a limited number of recipients of an allogeneic stem cell transplantation with hCMV reactivation prior to day 100, the median duration of hCMV-DNAemia in patients with reconstitution of IE1-specific and UL83/pp65-specific CD8 T cells was significantly shorter than in patients with reconstitution of UL83/pp65-specific CD8 T cells only.

The comparison of sorted IE1-TCR_{high} CD8^+ T cells versus the sum of the remaining IE1-TCR^− CD8^+ T cells has revealed an enrichment of protective cells in the IE1-TCR^+ subset (13). However, this cannot be taken to mean that IE1-specific CD8 T cells are superior, as they were enriched to ~100%, whereas CD8 T cells specific for the other known epitopes of mCMV (Fig. 11) stayed at ~1% among a majority of cells with specificities unrelated to mCMV. As shown in Fig. 13, the dose-response regression lines for the two subsets are not parallel but intersect at high cell numbers. The steeper slope observed for the IE1-TCR^− subset indicates a more efficient control at higher cell numbers. This is explained by the fact that in this subset viral epitope-specific cells become limiting at low cell numbers and that one frequent specificity, namely m164, dominates until the low-frequency specificities, namely m18, m04, M83, M45, and M84, come in and contribute to the protection at the high cell numbers. The conclusion that CD8 T cells specific for different epitopes can cooperate for protection is supported by the work of Ye et al. (62) who have documented an improved protection against challenge...
infection after coimmunization with plasmids expressing mCMV IE1 and M84. Since a cell presenting different viral epitopes cannot be killed twice, cooperative effects are more likely to indicate differential presentation of these epitopes on different cell types or in the kinetics of viral gene expression.

We have of course considered the approach to directly compare the antiviral function of sorted IE1-TCR$^+$ and m164-TCR$^+$ cells, but this experiment is pending because the Dd dimer is not yet on the market. Nevertheless, a comparison can be made at low cell numbers at which, as discussed above, m164 accounts for the protection by the IE1-TCR$^+$ subset.

While the enrichment by sorting predicted a 100-fold-higher protective activity of the IE1-TCR$^+$ cell population, the observed difference in protective activity was only $\approx$10-fold (Fig. 13). With some caution based on the fact that in this experiment only the IE1-specific cells were touched by TCR binding reagent, these findings suggest a higher efficacy of the D$^d$ restricted m164 (257-AGPPRYSRI-265) epitope-specific cells. This may relate to the previous finding that this peptide is constitutively presented during the E phase in infected cells (19) in the presence of all three immune evasion proteins of mCMV, namely, m04/gp34, m06/gp48, and m152/gp40 (for reviews, see references 16 and 45), whereas presentation of the IE1 peptide occurs in the E phase only in certain cell types (18) or under conditions of IFN-γ-induced enhancement of processing and presentation (17). As shown previously for an IE1-CTLL, the IE1 epitope-specific antiviral efficacy in an adoptive transfer and coinfection setting was indeed markedly improved against mutant virus mCMV-$\Delta$m152 compared to revertant virus mCMV-$\Delta$m152-rev (51).

In view of the susceptibility of IE1 peptide presentation to inhibition by the viral immune evasion proteins, the finding that as few as 400 sorted IE1-TCR$^+$ CD8$^+$ T cells were nonetheless sufficient to control mCMV-WT infection of adoptive transfer recipients by $\sim 1$ log$_{10}$ in the lungs, $\sim 3$ log$_{10}$ in the spleen, $>2$ log$_{10}$ in the liver, and $\sim 1$ log$_{10}$ in the adrenal glands is indeed astonishing and documents an outstanding antiviral efficacy of these cells. As shown in Fig. 7, a similar degree of protection required $10^5$ cells of a third-restimulation IE1-CTLL, that is, an $\sim$250-fold-higher number of cell culture-propagated CD8$^+$ T cells of the very same epitope specificity!

It must be emphasized that for these experiments we kept the in vitro cultivation and restimulation period to the minimum required for selecting a monospecific but polyclonal IE1-
CTL with a Vβ composition still closely resembling that of the starting CD8-TM population. Specifically, predominant usage of Vβ8.1-Vβ8.2 followed by Vβ10a and Vβ8.3 was fairly well conserved during the IE1 epitope-specific in vitro selection (Fig. 1 and 2). It is worth noting that T-cell clone IE1, the prototype clone with which the IE1 epitope was originally defined (46, 50), expressed a Vβ6 TCR (M. J. Reddehase, unpublished data). Previous studies of the Vβ expression by IE1 epitope-specific CD8 T cells also indicated a predominant usage of Vβ8 (54), specifically of Vβ8.1-Vβ8.2 (30) and have suggested preferential expansion of Vβ8.1-Vβ8.2 cells during an in vivo mCMV infection, leading to an increasing oligo-clonality over time (30). The Vβ usage distribution among polyclonal IE1 epitope-specific late CD8-TM cells observed in our experiments (Fig. 1) is in accordance with the data reported by Karrer et al. (30); yet, our interpretation with regard to the selection of the clonal repertoire during mCMV infection is somewhat different. Most relevant, the Vβ composition of the IE1 epitope-specific CD8-TM pool eventually mirrored the epitope-independent general Vβ composition of early and late memory pools and even the homeostatic Vβ composition of the CD8 T-cell pool in uninfected, unprimed BALB/c mice. It thus appears that Vβ usage frequencies among IE1 epitope-specific CD8 T cells reflect the genetically determined general frequencies. This becomes particularly evident from the conserved predominance of Vβ8.1/8.2 usage (Fig. 1 and 2). We thus conclude that the IE1 epitope-specific expansion in vivo as well as during several rounds of restimulation in vitro is broadly polyclonal and in its rate largely independent of the Vβ family of the TCRs. All in all, Vβ usage was not likely the determinant of in vivo antiviral efficacy in the experiments reported here.

From our own work in progress, we are well aware of the fact that the selection conditions in cell culture define the average TCR affinities of the resulting CTLs and that this may have an impact on the in vivo antiviral efficacy (D. Gillert-Marien, unpublished data). Likewise, the coreceptor molecule CD8 contributes to overall avidity and may enhance antiviral efficacy by stabilizing the interaction between the TCR and the MHC-peptide complex. As shown in Fig. 10, effector cell function in an IE1-CTL compared with the starting CD8-TM population was triggered by lower peptide concentrations. Thus, TCR affinity for the MHC-peptide complex and/or avidity of the interaction between effector cells and target cells was not the limiting factor.

In years of experience with generating CTLs and testing them for in vivo function in adoptive transfer experiments (22, 24, 25), we never observed protection with such low cell numbers as are shown here for the ex vivo-sorted cells. We therefore believe in a more fundamental difference between ex vivo recovered and in vitro selected and propagated epitope-specific CD8 T cells. We have not yet further pursued this phenomenon experimentally in order to provide the final explanation here, and it is evident that this is not a trivial issue to be studied in polyclonal natural immune responses, in which CD8 T cells specific for any particular epitope represent only a minor fraction. Clearly, these questions are much easier to address in TCR transgenic models (60). Obvious ideas include differences in the in vivo proliferative potential and survival time after transfer, as well as differences in lymphoid homing and tissue infiltration properties. Of likely importance is the difference in the differentiation stage of the transferred cells. While the IE1 epitope-specific, spleen-resident early CD8-TM cells tested in our experiments represented CD62Lhi central memory cells (CD8-TCM) and CD62Llo effector-memory cells (CD8-TEM) at a ratio of ~1:1, the cells of the IE1-CTL derived thereof after three rounds of in vitro restimulation and many more cell divisions were highly activated, cytolytically active CD62Llo effector cells. As it has been shown recently by Wherry et al. (60) with a TCR transgenic model, CD8-TCM cells, in comparison to CD8-TEM cells, have a greater capacity to persist in vivo and are more efficient in mediating protective immunity because of their increased proliferative potential. It is reasonable to predict that effector cells of a CTL have even less in vivo proliferative potential than the CD8-TEM cells. This may be the key to understanding the great difference in the protective capacities between sorted IE1 epitope-specific CD8-TM cells and IE1-CTLs observed here.

Early work with the BALB/c mouse model successfully using polyclonal, acutely primed lymph node CD8 T cells (49, 52, 58)
or spleen-resident CD8-TM cells (47) for adoptive transfer therapy.

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REFERENCES


