The Efficacy of Antigen Processing Is Critical for Protection against Cytomegalovirus Disease in the Presence of Viral Immune Evasion Proteins

Rafaela Holtappels, Doris Thomas, and Matthias J. Reddehase

Institute for Virology, University Medical Center of the Johannes Gutenberg University, 55131 Mainz, Germany

Received 12 May 2009/Accepted 19 June 2009

Cytomegaloviruses (CMVs) code for immunoevasins, glycoproteins that are specifically dedicated to interfere with the presentation of antigenic peptides to CD8 T cells. Nonetheless, the biological outcome is not an immune evasion of the virus, since CD8 T cells can control CMV infection even when immunoevasins are expressed. Here, we compare the processing of a protective and a nonprotective epitope derived from the same viral protein, the antiapoptotic protein M45 in the murine model. The data provide evidence to conclude that protection against CMVs critically depends on antigenic peptides generated in an amount sufficient to exhaust the inhibitory capacity of immunoevasins.

Cytomegalovirus (CMV) infection causes multiple organ disease in the immunocompromised host but is usually well controlled in the immunocompetent host. Experimental immunotherapy of CMV infection by adoptive cell transfer revealed a protective function of virus-specific CD8 T cells in the model of murine CMV (mCMV) infection (11, 14) as well as in clinical trials of a cytotoxic immunotherapy of human CMV infection (4, 19, 24, 28). Notably, as shown in the murine model, CMV-specific CD8 T cells protect in an epitope-specific, T-cell receptor-dependent manner (1), although both viruses code for multiple glycoproteins, so-called immunoevasins, that interfere with the major histocompatibility complex (MHC) class I pathway of antigen presentation (for a review, see reference 22). So, apparently, immunoevasins may indeed inhibit the trafficking of peptide-loaded MHC class I molecules but fail to completely prevent cell surface presentation.

While all but one of the currently known antigenic peptides of mCMV, as far as tested, mediate protection regardless of the numerical immunodominance of the cognate CD8 T cells (reviewed in reference 11), the MHC Dd-presented peptide 985-HGIRNASFI-993 (10) derived from the antiapoptotic protein M45 (3), briefly named here the M45-Dd peptide, is the prototype of a nonprotective mCMV epitope (12). The reason for this exceptional position is currently unknown. It appears to result from inefficient epitope presentation by the infected cells and not from a functional deficiency of the cognate effector cells, since M45-Dd epitope-specific ex vivo-sorted CD8 T cells as well as cytolytic T lymphocytes (CTL) propagated as CTL lines (CTLL) protected against infection with an mCMV mutant in which the gene encoding the major immunoevasin m152/gp40 (5, 18, 25, 31) was selectively deleted (16). Thus, immunoevasins are causally involved in determining whether or not a particular epitope is protective, a conclusion that is of considerable relevance in the choice of epitopes for vaccines and cytoimmunotherapy. One possible explanation to be discussed is that the M45-Dd complex is more efficiently retained by m152/gp40. Alternatively, inefficient generation of M45-Dd complexes may facilitate a complete blockade of epitope presentation by immunoevasins, while the generation of high numbers of MHC peptide complexes might exhaust the inhibitory capacity of the immunoevasins. Here we present evidence in support of the second alternative.

Although discussed previously (5, 13), the interrelation between the efficiency of antigen processing and immunoevasion function, and its implication for peptide presentation and antiviral protection, has not been elucidated experimentally. For comparing the nonprotective M45-Dd epitope with a protective epitope, we chose the protective M45-derived, Dd-restricted antigenic peptide 507-VGPALGRGL-515, briefly named the M45-Dd peptide (12) (Fig. 1A). The common protein source for both peptides avoids an influence of biochemical protein properties on differences in antigen processing and presentation. The immune response to mCMV in the corresponding mouse strains BALB/c (H-2^d) and C57BL/6 (H-2^b) is well characterized and has been reviewed recently (7, 14).

As shown in Fig. 1B by the frequencies of the M45 peptide-specific memory CD8 T cells, the immune response to the M45-Dd epitope in C57BL/6 mice is numerically higher than the response to the M45-Dd epitope in BALB/c mice, both in terms of absolute frequencies (Fig. 1B, left panel) and normalized to the total reactivity measured by sensitizing CD8 T cells polyclonally through the signal-transmitting T-cell-receptor-complex component CD3ε for gamma interferon (IFN-γ) secretion (Fig. 1B, right panel). This normalization is important because memory cell populations still contain a majority of naive precursor cells so that only a small fraction of ex vivo-isolated CD8 T cells are capable of responding in the assay (15, 16). So, with regard to the magnitude of the memory CD8 T-cell response, the M45-Dd epitope is immunodominant, while the M45-Dd epitope is subdominant.

We then generated the respective epitope-specific CTLL for
testing functional avidity in the cytolysis assay in vitro (Fig. 2A) and protective activity upon adoptive transfer in vivo (Fig. 2B). While M45-Dd CTL recognized peptide-loaded target cells with a half-maximal activity at a peptide concentration of \(10^{-10}\) M, M45-Db CTL reached half-maximal activity already between \(10^{-11}\) M and \(10^{-12}\) M. Despite the more than 10-fold higher functional avidity of M45-Dd CTL, the cells failed to control infection in vivo as shown exemplarily for the lungs, whereas M45-Dd CTL were protective despite their lower functional avidity (Fig. 2B). In conclusion, the functional avidities of the CTL can indicate the failure in protection by M45-Dd CTL.

So far in this work, peptides were loaded onto cell surface MHC class I molecules by the incubation of target cells with defined concentrations of synthetic peptide. Under infection conditions, peptide-loaded MHC class I molecules are generated within infected cells by antigen processing. This multistep process involves protein cleavage, which usually takes place in the proteasome and generates antigenic peptide precursors, the translocation of these precursors into the endoplasmic reticulum lumen via the transporter associated with antigen processing, N-terminal trimming to the final peptide size, and loading onto nascent MHC class I molecules in a specific loading complex (for reviews, see references 26 and 29). Differences in the efficacy of the processing process at any of these stages can lead to differences in the number of MHC peptide complexes generated in the infected cells. We have therefore quantitated the M45 peptides in chromatographically fractionated acidic lysates of infected cells (Fig. 3A). Since unbound peptides are degraded, the number of peptides isolated from a cell is equivalent to the number of peptide-loaded MHC class I complexes present in the cell (5, 9). In a first approach, antigenic peptides—and thus MHC peptide complexes—were quantitated in the absence of immunoevasin m152/gp40 after the infection of fibroblasts of the respective haplotype with the bacterial artificial chromosome-cloned deletion mutant mCMV-Am152 (27). Notably, whereas \(1,200\) M45-Dd peptides per cell were recovered, infected cells contained only \(2\)
M45-D\(^{b}\) peptides (Fig. 3A, top panels). This implies a very inefficient processing of M45-D\(^{b}\). The difference remained visible in the presence of immunoevasin m152/gp40 after infection with the bacterial artificial chromosome-cloned revertant...
virus mCMV-Δm152-rev (16), namely, ~5,800 M45-D\(^d\) peptides compared to ~11 M45-D\(^b\) peptides (Fig. 3A, bottom panels). The increase in the extractable amount of peptide in the presence of m152/gp40 may be explained by the accumulation of MHC peptide complexes in a cis-Golgi compartment, which is the known mechanism by which m152/gp40 interferes with peptide presentation (31).

For correlating intracellular processing with cell surface presentation in the absence and presence of the immunoevasin m152/gp40, peptide presentation was measured by the capacity of the infected cells to sensitize epitope-specific but still polyclonal M45-D\(^d\) and M45-D\(^b\) CTLL for IFN-\(\gamma\) secretion (Fig. 3B). For characterizing the two CTLL in this assay and with fibroblasts as the target cell type, the functional avidity distributions were determined by measuring the frequencies of effector cells sensitized by fibroblasts loaded with graded concentrations of the respective peptide (Fig. 3B, left panels). This confirmed the avidity difference between the two CTLL in that the threshold concentration for recognition was 10\(^{-10}\) M for M45-D\(^d\) and 10\(^{-11}\) M for M45-D\(^b\), with modal value concentrations of 10\(^{-9}\) M and 10\(^{-10}\) M, respectively. Despite lower functional avidity but in accordance with efficient antigen processing, a low but detectable number of cells of the M45-D\(^d\) CTLL recognized fibroblasts infected with mCMV-Δm152-rev expressing immunoevasin m152/gp40. Conversely, the M45-D\(^b\) CTLL failed despite higher functional avidity but in accordance with inefficient antigen processing. In contrast, a significant number of cells in both CTLL recognized fibroblasts infected with mCMV-Δm152 (Fig. 3B, right panels).

Finally, we correlated the recognition of infected fibroblasts in cell culture with in vivo protection (Fig. 4). Notably, the in vitro data for fibroblasts (Fig. 3B) were found to precisely predict protection in vivo. Specifically, M45-D\(^d\) CTL protected against both mutant and revertant virus but with a dose-response efficacy that was reduced in the presence of immunoevasin m152/gp40, leading to an ~50-fold difference in lung virus titers after the transfer of 10\(^6\) cells (Fig. 4, left panels). In contrast, M45-D\(^b\) CTL protected only when the immunoevasin gene was deleted (Fig. 4, right panels).

Remarkably, a steady-state level of very few M45-D\(^b\) complexes per cell led to a cell surface expression sufficient for target cell recognition and in vivo protection, provided that immunoevasins generally inhibit the transport of peptide-loaded MHC class I complexes to the cell surface, the completeness of this inhibition is critically influenced by the efficacy of antigen processing.
REFERENCES


