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Molecular aspects of immunological evasion by cytomegalovirus*

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Immune surveillance of CMV infection

Cytomegaloviruses (CMVs) are species-specific herpesviruses that constitute the Betaherpesvirus subfamily. Like all herpesviruses, they establish lifelong infection in their hosts despite the presence of an active immune system. Primary infection with human CMV (HCMV) in the immunocompetent host is usually asymptomatic and leads to the establishment of a latent state from which reactivation can occur. However, infection of the immunologically immature or immunodeficient host such as organ transplant recipients or AIDS patients, can cause severe and often fatal disease *(21)*. Analysis of the pathogenesis of the HCMV infection is hampered by the species specifity of cytomegaloviruses. Therefore, infection of the mouse with murine CMV (MCMV) is widely used as a model for HCMV infection and biology *(22)*.

CMV gene expression is regulated in a cascade fashion. Viral proteins expressed in the immediate-early (IE) phase of infection are required for the subsequent transcription of early (E) genes *(26)*. E proteins regulate the viral DNA replication, which is followed by the synthesis of the structural proteins during the late phase of infection.

Although different natural immunity mechanisms, especially NK cells, are associated with host resistance to CMV infection *(35)*, a specific immune response is necessary for the clearance of CMV. During infection specific antiviral antibodies are produced and specific T lymphocytes are activated. Antibodies are not essential for the control of the acute viral infection, but they prevent viral spread after reactivation of CMV from latency *(25)*. The presence of T lymphocytes is required for the clearance of the acute infection. $CD8^+$ T cells play a decisive role in the antiviral defence *(32, 33)*. They recognise peptides derived from viral proteins synthesised in infected cells and presented at the cell surface by specialised molecules encoded in the major histocompatibility complex (MHC) *(38)*. Despite their dominant role in the control of CMV infection, $CD8^+$ T lymphocytes are not indispensable. In their absence the virus is cleared by $CD4^+$ -subset-dependent mechanisms *(24)*. The antiviral activity of $CD4^+$ cells can be mediated either by the direct, MHC II restricted, cytotoxic potential of these cells or by the production of cytokines like IFN-gamma and TNF-alpha, that have an antiviral effect *(28, 29)*.

To establish a persistent infection in an immunocompetent host viruses require sophisticated mechanisms to struggle or to escape from the host immune control. CMV commands several mechanisms that enable the evasion form the host immune response. There is strong evidence that HCMV and MCMV possess several genes whose products interfere with immune effector mechanisms (Figure 1). More than one strategy is used. Viral proteins are expresed that bind to the Fc fragment of host immunoglobulins (Ig), others interfere with the antigen processing and presentation process in the MHC class I pathway. Additionally, HCMV encodes a homologue of the MHC class I heavy chain *(13)* and functional beta chemokine receptor *(17)*. Both proteins are thought to intervene with natural immunity mechanisms.

The potential role of the CMV Fc receptor in immune evasion

The CMV protein with IgG Fc binding properties is certainly one of the viral products that has an obvious potential to interfere with the humoral immunity. The binding of human IgG to HCMV infected cells has been described long ago *(27)* but the gene responsible for this phenomenon is still not localised. The MCMV gene coding for the Fc receptor (FcR)

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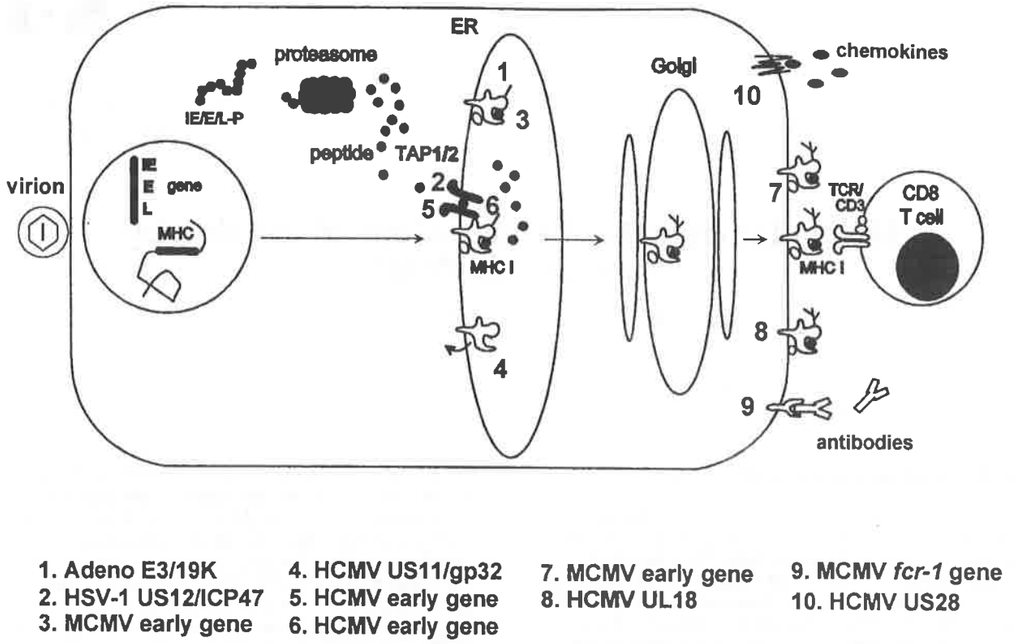

1. Adeno E3/19K
2. HSV-1 US12/ICP47
3. MCMV early gene
4. HCMV US11/gp32
5. HCMV early gene
6. HCMV early gene
7. MCMV early gene
8. HCMV UL18
9. MCMV *fcr-1* gene
10. HCMV US28

FIGURE 1. *Viral immune evasion mechanisms. Different mechanisms used by cytomegalovirus are shown. For comparison, similar functions found first in other viruses are also listed. Viral gene products modulated the intracellular transport of the MHC class I molecules (1, 3), the peptide transport form the cytosol into the ER (2, 5), the asssembly of the trimolecular class I complexes (4, 6), actively remove surface class I proteins (7), express a class I homolog and subsequently reduce NK sensitivity of the infected cells (8), bind host immunoglobulins (9) or serve as a receptor for chemokines (10).*

has recently been identified *(36)*. It was observed that Fc fragment of murine IgG can precipitate viral glycoproteins (gp) of 88 and 105 kDa from MCMV-infected cells. The corresponding gene was located by using a DNA microinjection technique. The strategy for identification of the viral gene and the characterisation of its product is shown of Figure 2. Undigested MCMV DNA, restriction enzyme-digested DNA, eluted DNA fragments and finally cloned restriction fragments were injected into the nuclei of cells. The expression of a protein binding to mouse IgG was determined by immunoflorescence. This approach led to the localisation of the gene in the HindIII J fragment of the MCMV genome. The correct identification of the gene was confirmed by expression of the gene in a recombinant vaccinia vector. Further studies have shown that the MCMV FcR is expressed at the cell surface of infected cells, but the expression is rather weak and that no Fc binding activity of the virion can be detected.

Although the immunoevasive function appears obvious, the functional relevance of the Fc binding property and the potential immunoevasive role of the FcR are still questionable. Several possibilities exist. On the basis of the homology to FcR of alphaherpesviruses, i.e. herpes simplex virus type 1 (HSV-1) gE, we can presume that the MCMV FcR, expressed at the surface of the infected cell, may protect them from antibody dependent cellular cytotoxicity (ADCC) and complement mediated lysis. This function was described for the HSV-1 gE and gI complex that form an Fc receptor *(12)*. The hypothetical mechanism was described as bipolar bridging *(14)*. After binding of the antibody with its $F(ab)_2$ fragment to viral antigens on the surface of the infected cell, the FcR interacts with the Fc part of the antibody molecule and should prevent its interaction with immune effector mechanisms that are activated through binding with Ig Fc fragment. The other possibility is that the FcR property only reflects the potential of this protein to interact with certain cellular glycoproteins, and that, like in the cases of HSV-1 FcR and PRV gI, this MCMV protein is involved in the direct cell to cell spread of the virus *(11, 43)*. These viral proteins are not required for infection of cells by extracellular virus, but they facilitate the direct cell to cell route, which is used during viral spread in some tissues and between certain cultured cells. This role of the

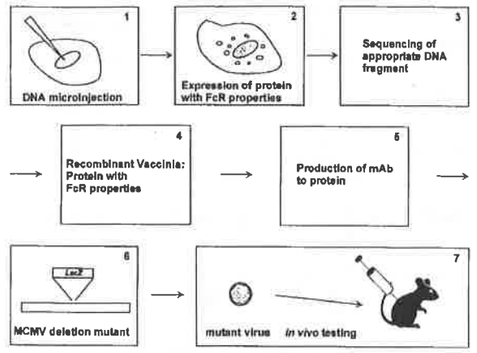

FIGURE 2. *Strategy for the identification and characterisation of the MCMV FcR gene. MCMV DNA was injected into the nuclei of the cells (1) and the expression of a protein with FcR properties was determined by immunofluorescence (2). After sequencing of DNA fragments (3), the correct idenfification of the gene was confirmed by expression in a recombinant Vaccinia vector (4). To study the function of the gene product it is necessary to produce specific monoclonal antibody (5) and a deletion mutant virus (6). The role of the FcR in viral replication and spread can be estimated after* in vivo *testing of the mutant virus and revertant (7).*

MCMV FcR is supported by the presence of a RGD motif in the amino acid sequence. This short sequence represent a ligand recognition motif for several integrins *(30)*.

In order to test the biological role of the FcR in a natural virus-host model we constructed a FcR deletion mutant (ΔMS94.4), disrupting the *fcr 1* gene by insertion of the *E coli lacZ* gene (Figure 3). The *in*

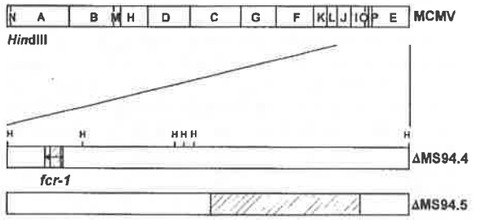

FIGURE 3. *Construction of the ΔMS94.4 (FcR⁻) and ΔMS94.5 recombinant viruses. Shown is the* HindIII *cleavage map of the MCMV genome (top) and the expanded region form* HindIII-J *to* HindIII-E *fragment (bottom) with* HindIII *cleaveage sites (H). The position of the* fcr-1 *gene is indicated by an arrow. The hatched boxes depict a 1,3 kb and a 15,8 kb fragment deleted in the ΔMS94.4 and in the ΔMS94.5 recombinant, respectively.*

vitro growth kinetic of recombinant virus is comparable to the wild type virus, indicating that the FcR is dispensable for viral growth in cell culture. Like some other CMV genes, *in vitro* nonessential, the *fcr-1* gene may be required for efficient replication in the infected host, for instance to evade humoral immunity. In order to test this presumption, we infected new-born Balb/c mice with the FcR^- recombinant and determined the virus titers 14 days after infection. Infection with ΔMS94.4 deletion mutant resulted in significantly reduced virus titers in different organs in comparison to wild type virus (Figure 4). Therefore,

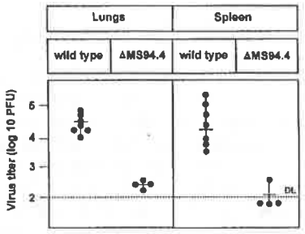

FIGURE 4. *The attenuated phenotype of FcR^- virus. New-born Balb/c mice were infected with $1x10^3$ PFU of wild type MCMV and ΔMS94.4 recombinant virus (FcR^-). Virus titers at day 12 post infection were determined for individual mice (closed circles) and median values (horizontal bars) were calculated.*

the loss of this gene was associated with a loss of virus fitness *in vivo*. Before making definitive conclusions about the role of the FcR for viral replication *in vivo*, it is necessary to create a FcR^+ rescued virus. The construction of herpesvirus mutants is often associated with unwanted illegitimate recombinations. Therefore the attenuated phenotype of the recombinant virus can be attributed to the loss of FcR only if it is possible to restore the wild type phenotype by reinsertion of the gene. Then the mechanisms by which MCMV FcR acts can be addressed. This will allow to connect the reduced virulence of the FcR^- virus either with the suggested loss of an immunoevasive function mediated by antibody binding, or with the unknown mechanisms that are important for viral spread *(10)*.

Interference with antigen presentation in the MHC class I pathway

HCMV and MCMV have the ability to interfere with antigen presentation in the MHC class I pathway *(1, 8)* (Figure 1). Despite the high degree of their genomic and biological similarity, different mechanisms are used that result in a similar phenotype.

Several laboratories have shown that HCMV down-regulates MHC class I molecules *(1, 2)*. In the infected cells the MHC class I assembly is reduced

and the degradation of the newly synthesised molecules is enhanced *(39, 42)*. The availability of HCMV deletion mutants revealed that a terminal genomic region contained more than one gene which independently down-regulate MHC class I heavy chain expression *(23)*. The HCMV US11 gene product down-regulates class I molecules expression by dislocating MHC heavy chains from the endoplasmic reticulum (ER) to the cytosol, where they are rapidly destroyed by the cytosolic enzymes *(41)*. A stable MHC complex is not formed. The second locus containing genes that affect MHC class I molecules is composed of the US2-US5 ORFs. The mechanism by which this gene(s) down-regulate MHC class I expression is not known.

Further, HCMV inhibits the TAP-dependent-transport of peptides from the cytosol into the ER. In the HCMV mutant ts9, in which the region between US1-US14 is deleted, TAP functions appear normal *(19)*. Therefore, the gene coding for this function is probably located in this genomic region. Remarkably, the inactivation of TAP was first described as a mechanism used by the HSV-1 ICP47 protein *(15, 20)*. By blocking the transport of cytosolic peptides into the lumen of the ER, ICP47 prevents the generation of stable MHC class I molecules and thus their ER export and expresion at the surface of the infected cell. Given the essential function of TAP in translocation of antigenic peptides in the ER, it is not unexpected that it is a target for more than one member of the herpesvirus family. The unknown HCMV gene affecting TAP certainly differs from the HSV-1 ICP47 because no ICP47 homolog has been detected in the sequence of the HCMV genome.

MCMV interacts with the class I antigen presentation pathway by a different mechanism. The dominant MCMV antigen, recognised by the majority of antiviral cytotoxic T lymphocytes (CTL), is a nonapeptide derived form the IE protein pp89 and presented by L^d MHC class I molecules. It was observed that L^d -restricted pp 89 specific CTL clone recognises pp89 during the IE phase of infection, but not during the early phase *(7, 31)*. Lack of CTL recognition after expression of the E genes suggested the presence of a viral gene(s) that interact wiht antigen presentation. The rate of synthesis, stability and nuclear transport of pp89 was found no to be altered during the E phase. Neither were the formation and the stability of the trimolecular complex of MHC class I heavy chain, β_2-microglobulin and peptide affected. Studies on the glycosylation pattern of MHC class I molecules revealed that they remained in an Endo H-sensitive form. This observation suggested that the complex is not degraded as in HCMV infected cells, but is retained in the endoplasmic reticulum (ER)/cis-Golgi compartment *(9)* (Figure 5).

The first step toward the indentification of the genes whose products are responsible for retention of MHC class I molecules, was the construction of a recombinant MCMV deletion mutant that differs form wild type virus in its effect on antigen presentation. In the design of the deletion mutant we were directed by the sequence analysis of the MCMV genome. The

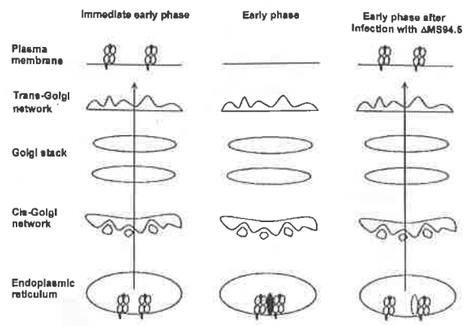

FIGURE 5. *The MCMV early gene product retains MHC class I molecules in the ER/cis-Golgi compartment. During the immediate early phase class I molecules are transported to the celll surface. In the early phase class I molecules are retained in the ER/cis-Golgi cellular compartment. The putative viral gene product responsible for this effect is symbolised by the closed oval. In the Δ MS94.5 recombinant virus this gene is deleted, and transport of the MHC molecules to the cell surface is restored.*

comparison between herpesvirus genomes has revealed conserved gene blocks shared between alpha-, beta- and gammaherpesviruses. The conserved gene blocks are located in the central portion of the genome and probably encode essential functions. The genes in the termini of the genomes are not conserved between different herpesviruses and are probably not essential for replication *in vitro*. It was reasoned that gene(s) involved in retention of a MHC class I molecules are members of one of the gene families located in the termini of the viral genome, similar to those encoding the HCMV US families. Therefore, the MCMV deletion mutant ΔMS94.5 was constructed with a large deletion (15,8 kb) in the genomic area represented by the *Hind*III E fragment of the MCMV genome (Figure 3) *(37)*. The replication kinetics of the recombinant virus under *in vitro* conditions was indistinguishable from that of wild type virus. The next step was to verify if the viral genes that cause the block of MHC class I molecules transport are deleted in mutant ΔMS94.5. Therefore we compared the glycosylation pattern of MHC class I molecules in cells infected with wild type and recombinant virus. After infection with wild type virus and expression of E genes, MHC molecules remain Endo H-sensitive, typical for ER/cis-Golgi glycoproteins. In contrary, after infection with the ΔMS94.5 deletion mutant under the same conditions, the glycosylation pattern of newly synthesised MHC class I molecules was identical to that of noninfected cells (Figure 5).

Also, the presentation of pp89 to specific CTLs in the E phase was restored in the ΔMS94.5 infected cells. Using conditions permissive for E-gene ex-

pression, in contrary to wild type MCMV, pp 89 presentation in the E phase and lysis by specific $CD8^+$ T cells was not abolished after infection with ΔMS94.5 (Figure 6).

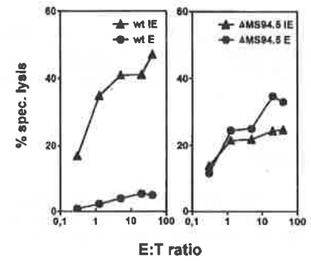

FIGURE 6. *Restored antigen presentation during the E phase in MS94.5-infected cells. Mouse embryonal fibroblast target cells were infected with wild type MCMV or with the MS94.5 recombinant virus. Antigen presentation of pp89 was tested by pp89-specific CTLs in a standard 4 h cytolytic assay under the infection conditions with selective expression of IE or IE and E viral genes.*

The results of the cytolytic assay as well as the glycosylation pattern of MHC class I molecules in cells infected with the ΔMS94.5 suggested that viral gene(s) responsible for retention were indeed deleted in recombinant ΔMS 94.5. The next step in our experiments is the detailed analysis of the responsible gene that we have already defined by the gene microinjection technique. This will allow us to study the molecular mechanism of the MHC class I molecules retention.

There is evidence for more than one early-gene function of MCMV, which affect the MHC class I pathway of antigen presentation. Namely, within 3-6 hours after MCMV infection surface class I molecules disappear from the membrane of the infected cells. The export block of nascent MHC I complexes alone cannot explain this rapid reduction because the half life of resident class I molecules is much longer. It seems that, in addition to the retention of nascent class I molecules in ER/cis-Golgi, plasma membrane resident molecules are actively removed from the surface of the infected cells. The gene(s) that carry out this function are still unknown. They must be located outside the 15,8 kb gene region that is deleted in ΔMS94.5 and expressed in the E phase later than genes from this gene region because surface reduction of surface MHC complexes in present also in ΔMS94.5 mutant infected cells at later stages of infection. Viral E proteins that reside at the plasma membrane and coprecipitate with MHC class I molecules have been identified as potential candidates for this function. Whether they are really involved in the

MCMV effect on the class I molecules still has to be proven. Moreover, a reduction of the class I heavy chain synthesis has been proposed as additional mechanism of MCMV to affect antigen presentation *(4)*.

The presence of multiple genes that can interact with the MHC class I antigen presentation pathway is so far unique for CMV. They may be descendants of an unique ancestor gene, and may have resulted from duplication and divergence during the evolution process. This hypothesis is supported by the presence of the tandemly arranged glycoprotein gene families in the CMV genome *(5)*. However, a common origin of HCMV and MCMV MHC class I interacting genes is not likely, because beyond the phenotype of MHC class I reduction there is no significant homology between the genes and the products probably act by quite different mechanism.

The interference of MCMV with the transport of newly assembled MHC complexes combined with the removal of preexiting surface MHC class I proteins, represents a very effective evasion mechanism from $CD8^+$ T lymphocyte control. If we consider the role $CD8^+$ T cells still play in the control of the CMV infection, it is not surprising that MCMV has developed more than one function to evade CTL mediated control in order to persist in its host and be effectively transmitted despite the presence of an active immune system.

At the same time the still important role of $CD8^+$ T cells indicates that the viral mechanism of immune evasion do operate *in vivo* only with a limited efficacy. The host immune system has found ways to recognise and to eliminate infected cells via CD8-dependent mechanisms, despite viral functions that downregulate antigen presentation by MHC class I molecules. One of the mechanisms involved is represented by IFN-gamma. IFN-gamma upregulates expression of the MHC class I molecules and the excess of the MHC molecules can escape the transport block and present antigen at the plasma membrane *(18)*. $CD8^+$ T cells also release IFN-gamma and restore antigen presentation in a paracrine fashion. This observation reconciles findings of altered *in vitro* presentation of viral antigens by class I molecules in parallel with $CD8^+$ T cell mediated, MHC class I restricted protection *in vivo*. To what extent different viral mechanisms really contribute to CMV immunological evasion, can only be answered after *in vivo* studies of virus mutants that lack each of the MHC-reactive genes alone or in combination. This is a task that is possible to achieve only with MCMV recombinants.

Protection of the NK cells mediated lysis - a role for the viral class I homolog?

Although the reduction in MHC class I surface expression clearly results in a reduced T cell recognition of infected cells and probably has a positive effect for the multiplication and survival of CMV, it may also have some drawbacks for the virus. Namely, re-

duced MHC class I expression is balanced by enhanced NK sensitivity. It is known that NK cells are inhibited by the recognition of peptide filled class I molecules *(6)*. This may explain the significant role of NK cells in MCMV clearance *(40)*. The potential disadvantage of increased NK sensitivity due to the loss of class I molecules may be compensated by other viral mechanisms that counteract the NK-function. HCMV gene UL18 codes for a MHC class I homolog that binds cellular β_2-m and can present endogenous peptides *(13)*. It has been assumed that the peptide filled viral class I homolog which is expressed at the cell surface might engage NK cells inhibitory receptors and thus prevent the NK-mediated lysis of the infected cell. The UL18 gene product may represent the principle by which the virus balances the negative effects of MHC class I molecules down-regulation.

The HCMV chemokine receptor homologan additional mechanism of immune evasion?

Chemokines are small proinflamatory peptides with leukocyte-chemoattractant activity. The chemokine receptors are members of the G protein-coupled receptor superfamily. A data base search has revealed that the HCMV US28 gene product is 33% identical to the mammalian leukocyte receptors for alpha and beta chemokines *(16)*. There is also functional homology. The binding of the chemokine to the US28 gene product leads to the intracellular calcium mobilisation, but, distinct from that of known mammalian receptors, the binding is selective for beta chemokines *(17)*. It can be expected that expression of this protein may interfere with the host antiviral response but the definitive active principle is still enigmatic.

Concluding remarks

CMV is another example of a virus which has evolved multiple mechanisms to evade hosts immune control. Remarkably the strategies appear different from those employed by another DNA virus, Pox virus *(34)*. Although the viral interference with expression of MHC class I molecules is the best documented, CMV also modulates the humoral immune response as well as mechanisms of natural immunity. The CMV genome contains numerous genes whose functions are probably not essential for viral growth in fibroblasts *in vitro*. These genes demonstrate their effect only *in vivo*. Therefore we anticipate that all deletion mutants will show some degree of attenuation, either generally or only in some tissues of the host. The characterisation of the attenuation mechanism may cause formidable difficulties. Deletion of the genes coding for immune evasion mechanism as well as the genes coding for functions that participate in the infection of certain target cells, virus spread, regulation of gene expression or establishment of latency, will result in a similar phenotype: limited virus growth after infection.

These genes remain to be discovered. The dissection and analysis of their functions will provide us informations on new principles in cell biology and moreover, they hold the promise for new therapeutic approaches.

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REFERENCES

1. BEERSMA M F C, BIJLMAKERS M J E, PLOEGH H L 1993 Human cytomegalovirus down-regulates HLA class I expression by reducing the stability of class I heavy chains. *J Immunol 151:* 4455-4464
2. BROWNE H, SMITH G, BECK S, MINSON T 1990 A complex between the MHC class I homologue encoded by human cytomegalovirus and β_2 microglobulin. *Nature (London) 347:* 770-772
3. BURGERT H G, KVIST S 1985 An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. *Cell 41:* 987-997
4. CAMPBELL A E, SLATER J S 1994 Down-regulation of major histocompatibility complex class I synthesis by murine cytomegalovirus early gene expression. *J Virol 68:* 1805-1811
5. CHEE M S, BANKIER A T, CECK S, BJOHNI R, BROWN C M, CERNY R, HORSNELL T, HUTCHISON C A, KOUZARIDES T, MARTIGNETTI T A, PREDDIE E, SATCHWELL S C, TOMLINSON P, WESTON K M, BARELL B G 1990 Analysis of the protein-coding content of the sequence of the human cytomegalovirus strain AD 169. *Curr Top Microbiol Immunol 154:* 125-169
6. CORREA I, RAULET D H 1995 Binding of the diverse peptides to MHC class I molecules inhibits target cell lysis by activated natural killer cells. *Immunity 2:* 61-71
7. DEL VAL M, VOLKMER H, ROTHBARD J B, JONJIĆ S, MESSERLE M, SCHICKEDANZ J, REDDEHASE M J, KOSZINOWSKI U H 1988 Molecular basis for cytolytic T lymphocyte recognition of murine cytomegalovirus immediate-early protein pp89. *J Virol 62:* 3965-3972
8. DEL VAL M, MÜNCH K, REDDEHASE M J, KOSZINOWSKI U H 1989 Presentation of CMV immediate-early antigen to cytolytic T lymphocytes is selectively prevented by viral genes expressed in the early phase. *Cell 56:* 305-315
9. DEL VAL M, HENGEL H, HÄCKER H, HARTLAUB U, RUPPERT T, LUČIN P, KOSZINOWSKI U H 1992 Cytomegalovirus prevents antigen presentation by blocking the transport of peptide-loaded major histocompatibility complex class I molecules into the medial-transport of peptide-loaded major histocompatibility complex class I molecules into the medial. Golgi compartment. *J Exp Med 176:* 729-738
10. DINGWELL K S, BRUNETTI C R, HENDRICKS R L, TANG Q, TANG M, RAINBOW A J, JOHNSON D C 1994 Herpes simplex virus glycoproteins E and I facilitate cell-to-cell spread *in vivo* and across junctions of cultured cells. *J Virol 68:* 834-845
11. DINGWELL K S, DOERING L C, JOHNSON D C 1995 Glycoproteins E and I facilitate neuron-toneuron spread of herpes simplex virus. *J Virol 69:* 7087-7098
12. DUBIN G, SOCOLOF E, FRANK I, FRIEDMAN H M 1991 Herpes simplex virus type I Fc receptor protect infected cells form antibody-dependent cellular cytotoxicity. *J Virol 65:* 7046-7050

13. FAHNESTOCK M, JOHNSON J L, FELDMAN R M R, NEVEU J M, LANE W S, BJORKMAN P J 1995 The MHC class I homolog encoded by human cytomegalovirus binds endogenous peptides. *Immunity 3:* 583-590
14. FRANK I, FRIEDMAN H M 1989 A novel function of the herpes simplex virus type 1 Fc receptor: participation in biploar bridging of antiviral imunoglobulin G. *J Virol 63:* 4479-4488
15. FRÜH K, AHN K, DJABALLAH H, SEMPE P, VAN ENDERT P M, TAMPE R, PETERSON R A, YANG Y 1995 A viral inhibitor of peptide transporters for antigen presentation. *Nature (London) 375:* 415-418
16. GAO J L, KUHNS D B, TIFFANY H L, McDERMOTT D, LI X, FRANCKE U, MURPHY P M 1993 Structure and functional expression of the human macrophage inflammatory protein 1 alpha/RANTES receptor. *J Exp Med 177:* 1421-1427
17. GAO J L, MURPHY P M 1994 Human cytomegalovirus open reading frame US28 encodes a functional beta chemokine receptor. *J Biol Chem 269:* 28539-28542
18. HENGEL H, LUČIN P, JONJIĆ S, RUPPERT T, KOSZINOWSKI U H 1994 Restoration of cytomegalovirus antigen presentation by gamma interferon combats viral escape. *J Virol 68:* 289-297
19. HENGEL H, FLOHR T, HÄMMERLING G J, KOSZINOWSKI U H, MOMBURG F 1996 Human cytomegalovirus inhibits peptide translocation into the endoplasmic reticulum for MHC class I assembly. *J Gen Virol (in press)*
20. HILL A, JUGOVIĆ P, YORK I, RUSS G, BENNIK J, YEWDELL J, PLOEGH H, JOHNSON D 1995 Herpes simplex virus turns off the TAP to evade host immunity. *Nature (London) 375:* 411-415
21. HO M 1994 Advances in understanding cytomegalovirus infection after transplation. *Transplant Proc 26:* 7-11
22. HUDSON J B 1979 The murine cytomegalovirus as a model for the study of viral pathogenesis and persistent infection. *Arch Virol 62:* 1-29
23. JONES T R, HANSON L K, SUN L, SLATER J S, CAMPBELL A E 1995 Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *J Virol 69:* 4830-4841
24. JONJIĆ S, PAVIĆ I, LUČIN P, RUKAVINA D, KOSZINOWSKI U H 1990 Efficacious control of cytomegalovirus infection after long-term depletion of $CD8^+$ T lymphocytes. *J Virol 64:* 5457-5464
25. JONJIĆ S, PAVIĆ I, POLIĆ B, CRNKOVIĆ I, LUČIN P, KOSZINOWSKI U H 1994 Antibodies are not essential for the resolution of primary cytomegalovirus infection but limit dissemination of recurrent virus. *J Exp Med 179:* 1713-1717
26. KEIL G M, EBELING-KEIL A, KOSZINOWSKI U H 1984 Temporal regulation of the murine cytomegalovirus transcription and mapping of viral RNA synthesised at immediate early times after infection. *J Virol 50:* 784-795
27. KELLER R, PEITCHEL R, GOLDMAN J N, GOLDMAN M 1976 An IgG-Fc receptor induced in cytomegalovirus infected human fibroblasts. *J Immunol 116:* 772-777
28. LUČIN P, PAVIĆ I, POLIĆ B, JONJIĆ S, KOSZINOWSKI U H 1992 Gamma interferon-dependent clearance of cytomegalovirus infection salivary gland. *J Virol 66:* 1977-1984
29. PAVIĆ I, POLIĆ B, CRNKOVIĆ I, LUČIN P, JONJIĆ S, KOSZINOWSKI U H 1993 Participation of endogenous tumour necrosis factor alpha in host resistance to cytomegalovirus infection. *J Gen Virol 74:* 2215-2223
30. PIERSCHABCHER M D, RUOSLAHTI E 1984 Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature (London) 309:* 30-33
31. REDEHASE M , FIBI M R, KEIL G M, KOSZINOWSKI U H 1986 Late-phase expression of a murine cytomegalovirus immediate-early antigen recognised by cytolytic T lymphocytes. *J Virol 60:* 1125-11129
32. REDDEHASE M J, MUTTER W, MUNCH , BUHRING H J, KOSZINOWSKI U H 1987 CD8 positive T lymphocytes specific for murine cytomegalovirus immediate-early genes mediate protective immunity. *J Virol 61:* 3102-3108
33. RIDDEL S, WATANABE K, GOODRICH J, LI C, AGHA C R, GREENBERG P 1992 Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. *Science 257:* 238-241
34. SMITH G L 1993 Vaccinia virus glycoproteins and immune evasion. *J Gen Virol 74:* 1725-1740
35. SHELLAM G R, ALLEN J E , PAPADIMITROU J M, BANCROFT G J 1981 Increased susceptibility to cytomegalovirus infection in beige mutant mice. *Proc Natl Acad Sci USA 78:* 5104-5113
36. THÄLE R, LUČIN P, SCHEIDER K, EGGERS M, KOSZINOWSKI U H 1994 Identification and expression of a murine cytomegalovirus early gene coding for and Fc receptor. *J Virol 68:* 7757-7765
37. THÄLE R, SZEPAN U, HENGEL H, GEGINAT G, LUČIN P, KOSZINOWSKI U H 1995 Identification of the mouse cytomegalovirus genomic region affecting major histocompatibility complex class I transport. *J Virol 68:* 6098-6105
38. TOWSEND A, BODMER H 1989 Antigen recognition by class I-restricted T lymphocytes. *Annu Rev Immunol 7:* 601-624
39. WARREN A P, DUCROQ D H, LEHNER P J, BORYSIEWICZ L K 1994 Human cytomegalovirus-infected cells have unstable assembly of major histocompatibility complex class I complexes and are resistant to lysis by cytotoxic T lymphocytes. *J Virol 68:* 2822-2829
40. WELSH R M, BRUBAKER J O, VARGAS-CORTES M, O'DONELL C L 1991 Natural killer (NK) cell response to virus infections in mice with severe combined immunodeficiency. The stimulation of NK cells and NK cell dependent control of virus infection occur independently of T and B function. *J Exp Med 173:* 1053-1063
41. WIERTZ J H J, JONES T R, SUN L, BOGYO M, GEUZE H J, PLOEGH H L 1996 The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell 84:* 769-779
42. YAMASHITA Y, SHIMOKATA K, SAGA S, MIZUNO S, TSURMI T, NISHIYAMA Y 1994 Rapid degradation of the heavy chain of class I major histocompatibility comlex antigens in the endoplasmic reticulum of human cytomegalovirus-infected cells. *J Virol 68:* 7933-7943
43. ZSAK L, ZUCKERMANN F, SUGG N, BEN-PORAT T 1992 Glycoprotein gI of pseudorabies virus promotes cell fusion and virus spread via direct cell-to-cell transmission. *J Virol 66:* 2316-2325

ABSTRACT

Molecular aspects of immunological evasion by citomegalovirus

Several viruses have developed strategies to modulate or evade the immune control of their hosts. Different ways of interference with distinct host mechanisms which contribute to the cotrol of the viral infection are used: from the interaction with complement system and cytokines, to the inhibition of the specific humoral and cellular immune response. Here, we describe principles of immune evasion used by cytomegaloviruses. They include gene products that bind host immunoglobulins, interfere with antigen presentation in the MHC class I pathway, abrogate the recognition by NK cells or serve as receptors for chemokines.

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