The Major Histocompatibility Complex Class II-linked *cim* Locus Controls the Kinetics of Intracellular Transport of a Classical Class I Molecule

By Simon J. Powis, Jonathan C. Howard, and Geoffrey W. Butcher

From the Department of Immunology, Agricultural & Food Research Council, Institute of Animal Physiology & Genetics Research, Cambridge Research Station, Babraham, Cambridge CB2 4AT, UK

Summary

The dominant *trans*-acting major histocompatibility complex (MHC)-linked class I modifier (*cim*) locus, previously recognized through its ability to determine altered alloantigenicity of a rat class I molecule, RT1.A^a, is shown here to influence class I intracellular transport. The MHC recombinant laboratory rat strains PVG.R1 and PVG.R8 display unusually long retention of RT1.A^a within the endoplasmic reticulum or *cis*-Golgi. In appropriate F_1 hybrid cells heterozygous for RT1.A^a and another class I MHC allele, RT1.A^c, only the RT1.A^a protein is subject to slow transport. The *cim* gene product therefore shows class I allele specificity in its action. *cim* appears to be a polymorphic locus whose product is directly involved in the processes of class I MHC assembly and/or intracellular transport.

Class I MHC antigens are highly polymorphic cell surface molecules that present endogenously derived peptide antigens to effector T cells of the immune system (1–3). They consist of a M_r 45,000 transmembrane heavy chain noncovalently associated with the M_r 12,000 β_2-microglobulin (β_2m)[1]. During biosynthesis the MHC class I heavy chains are inserted into the endoplasmic reticulum (ER), where N-linked core glycosylation of the heavy chain and association with β_2m occur (4). The loading of the class I molecule with suitable endogenous antigenic peptides may also occur at this early stage within the environment of the ER, possibly playing an important role in the genesis of the correct overall structure of the molecule (5–8). The assembled molecule is subsequently transported through the Golgi apparatus, where processing of the oligosaccharide side chains takes place (9), and is finally expressed at the cell surface, where presentation of the antigenic peptide occurs.

While the order of assembly of the component subunits of MHC class I, i.e., heavy chain, β_2m, and peptide, remains unclear, genetic data imply the involvement of other proteins in the process. MHC-linked regulatory loci that can influence assembly, transport, alloantigenicity, and antigen-presenting capacity of class I molecules have been reported in human, mouse, and rat systems (10–16). In our recent description of the rat *cim* system, we reported that recombination between the rat class I *RT1.A* region and the class II *RT1.B* region in PVG.R1 ($A^aB^cD^cC^c$) and PVG.R8 (A^a-$B^uD^uC^u$) recombinant rats resulted in the altered antigenicity of the RT1.A^a antigen (16). The *trans*-acting locus involved, *cim*, mapping close to the class II *RT1.B* region (16a) determined the expression of two alloantigenic forms of the RT1.A^a molecule, namely A^{a+} and A^{a-}, in the presence of the *cim*^{a} (dominant) and *cim*^{b} (recessive) alleles, respectively. A preliminary experiment indicated that the biosynthesis of the two forms also differed.

We describe here the control *cim* exerts on the biosynthesis of RT1.A^a both in lymphocytes from the recombinant rat strains and also in rat and mouse cell lines transfected with a cDNA encoding RT1.A^a, and discuss the possible modes of action of this novel MHC locus in relation to the recent identification of two genes mapping to the same region as *cim*, and which are homologous to the ATP-binding cassette family of membrane transporter proteins (17, 18).

Materials and Methods

Animals. All rats were bred and maintained in the Immunology Department, Babraham, Cambridge. The MHC haplotypes of the strains used are given in Table 1.

Media. Cells were maintained in RPMI 1640 (Flow Laboratories, Irvine, UK) supplemented with 5% FCS (Imperial Laboratories, Andover, UK). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Monoclonal Antibodies. Of the three rat anti-RT1.A^a mAbs used in this study, R3/13 (IgG2b) and R2/15S (IgG2a) are alloantibodies derived from AO anti-DA (*RT1^u* anti-*RT1^a*) immuniza-

[1] *Abbreviations used in this paper:* β_2m, β_2-microglobulin; endo H, endoglycosidase H; ER, endoplasmic reticulum; SR, spontaneous release; TfR, transferrin receptor.

tions, while MAC 30 (IgG2c) was derived from a PVG-*RT1*^{u} anti-PVG.R8 immunization. R3/13 recognizes the P site, and R2/15S and MAC 30 recognize the S site of RT1.A^a (19). The anti-RT1.A^c mAb YR5/12 (IgG2b) was obtained from an AO anti-PVG (*RT1*^{u} anti-*RT1*^{c}) immunization. All these mAbs are listed in reference 20. The mouse anti-rat class I mAb MRC OX-18 was also used (21). The mouse anti-rat transferrin receptor mAb MRC OX-26 (22) was a gift from Dr. A. Williams, MRC Cellular Immunology Unit, Oxford, UK.

Transfectant Cell Lines. The transfection of murine L cell fibroblasts with the RT1.A^a cDNA 3.3/1 has been described previously (23). The C58 cell line (full name, W/FuC58NT/D) is derived from a thymoma induced in a Wistar/Furth rat; the history of this cell line is detailed in reference 24. For C58 cells, the 3.3/1 cDNA was subcloned into the eukaryotic expression vector pMSD and the cells transfected by electroporation with pMSD 3.3/1 and the selection plasmid pMSD-HGPRT. Cells were selected in medium containing hypoxanthine, aminopterin, and thymidine (HAT), and sorted for expression of RT1.A^a by flow cytometry.

Pulse Chase Labeling. Con A lymphoblasts, generated by incubation of lymph node cells for 48 h at 2–3 × 10^6 cells/ml in RPMI, 5% FCS containing 5 μg/ml Con A (Sigma Chemical Co., Poole, UK), were labeled for 10 min with 25–50 μCi L-[^{35}S]methionine (Amersham International, Bucks, UK) after a 30-min preincubation in methionine-free MEM (Gibco Laboratories, Paisley, UK). Incorporation was terminated by the addition of a 10- to 20-fold excess of unlabeled methionine. Aliquots of cells were removed at the indicated timepoints, the cells pelleted, and immediately lysed in 200 μl of lysis buffer (2% [vol/vol] NP-40, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM PMSF, 20 mM Tris-HCl, pH 8.0). After a 30-min incubation on ice, the lysates were spun at 11,600 *g* for 10 min to remove debris, and stored at −20°C until immunoprecipitation.

Immunoprecipitation. Immunoprecipitations of class I MHC molecules were performed with antibodies coupled to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Cell lysates were precleared for 1 h at 4°C with 20 μl (packed volume) of Sepharose coupled with an irrelevant mAb. RT1.A^a was then isolated with 20 μl of R3/13-Sepharose or MAC 30-Sepharose for 1–2 h at 4°C. In sequential immunoprecipitations of class I molecules from the same lysate, after isolation of the RT1.A^a signal, 20 μl of MRC OX-18-Sepharose was added or, for RT1.A^c, 5 μg of affinity purified YR5/12 was added to the lysate for 45 min followed by 20 μl of sheep anti-rat Ig-Sepharose. The immunoadsorbents were then washed twice in buffer (0.5% [vol/vol] NP-40, 0.5 M NaCl, 10 mM EDTA, 100 mM Tris-HCl, pH 8.0) and boiled for 2 min in 20 μl of SDS sample buffer (2.3% [vol/vol] SDS, 5% [vol/vol] 2-ME, 10% [vol/vol] glycerol, 0.6 M Tris-HCl, pH 6.8).

For the immunoprecipitation of the rat transferrin receptor, cell lysates were precleared with 50 μl formalin-fixed *Staphylococcus aureus* cells (10% [vol/vol]), followed by the addition of 150 μl MRC OX-26 tissue culture supernatant for 1 h at 4°C. A further 50 μl of *S. aureus* cells was then added for 1 h at 4°C, and the immune complexes were washed three times in buffer (0.5% [vol/vol] NP-40, 150 mM NaCl, 5 mM EDTA, and 50 mM Tris-HCl, pH 7.4), followed by boiling in sample buffer as above.

Samples were analyzed by SDS-PAGE on 11% gels. These were treated with Amplify (Amersham International), dried, and fluorographed at −70°C.

Endoglycosidase H Digestion. After washing, the immunoadsorbents, in a volume of 20 μl, were supplemented with 2 μl of a 10% (vol/vol) stock of 2-ME and 4 μl of a 0.1% (wt/vol) SDS solution. They were then boiled for 1 min and cooled on ice. 8 mU of endoglycosidase H (endo H) (Boehringer Mannheim, Lewes, UK) was then added (control samples receiving PBS) and the digestion allowed to proceed at 37°C for 14 h. Digestion was terminated by the addition of 15 μl of SDS sample buffer and boiling for 2 min. Samples were analyzed by SDS-PAGE as above.

Flow Cytometry. Transfectant cells were distributed at 5 × 10^5 cells/well in a 96-well round-bottomed microtiter plate in a volume of 50 μl of PFN (PBS, 0.1% sodium azide, 2% FCS). 50 μl of mAb supernatant of R3/13, R2/15S, and MAC 30 was then added for 45 min at 4°C. Plates were washed three times with 200 μl/well PFN by centrifugation, and the cell pellets resuspended in 50 μl of FITC rabbit anti-rat Ig (Dako Ltd., Bucks, UK) for 45 min at 4°C. The plates were washed as before and the cells fixed in a 1% (vol/vol) formaldehyde solution. Flow cytometry was performed on a FACScan® (Becton Dickinson & Co., Mountain View, CA) with 10,000 cells being analyzed per sample.

Cytotoxic T Cell Assays. CTL assays were performed as previously described (16). Briefly, cytotoxic responses against the cell surface A^{a+} and A^{a-} forms of RT1.A^a were generated in MLC using cells from primed animals. PVG.R1 and PVG.R19 animals were primed against A^{a+} and A^{a-}, respectively, by injection of a lymph node cell suspension in PBS, 0.1 ml being injected into five subcutaneous sites on the back. Lymph node responder cells from primed animals were then cultivated with 2,000-rad gamma-irradiated stimulator cells (^{137}Cs source; Atomic Energy of Canada, Ottawa, Canada) in 200 μl of medium at a concentration of 1.5 × 10^6 responder cells/ml and 10^6 stimulator cells/ml in 96-well plates. The medium was supplemented with 10% rat Con A supernatant, 25 mM α-methyl mannoside (Sigma Chemical Co.), and 2.5 × 10^{-5} M 2-ME. Cells were incubated for 5 d, harvested, and assayed for cytolytic activity.

Target Con A lymphoblasts and transfectant cell lines were labeled with 50 μCi of sodium ^{51}Cr-chromate (Amersham International) for 1 h at 37°C, washed three times in RPMI, 5% FCS, and counted.

Effector cells were counted and adjusted to 1.5 × 10^7 cells/ml, and serial threefold dilutions prepared. 100 μl of effector cells plus 100 μl of labeled targets at 10^5 cells/ml were dispensed into microtiter wells. Control wells for spontaneous release (SR) values

Table 1. *Details of MHC Subregions of Strains Used in this Study*

Strain	*RT1* haplotype	*RT1* subregions *A*	*B*	*D*	*C*	*cim* allele*
Independent inbred						
PVG	*c*	*c*	*c*	*c*	*c*	*b*
MHC congenic						
PVG-*RT1*^{l}(LEW)	*l*	*l*	*l*	*l*	*lv1*	*a*
MHC recombinants						
PVG.R1	*r1*	*a*	*c*	*c*	*c*	*b*
PVG.R8	*r8*	*a*	*u*	*u*	*u*	*b*
PVG.R19	*r19*	*a*	*a*	*a*	*c*	*a*
PVG.R20	*r20*	*c*	*c*	*c*	*av1*	*b*

* *cim* maps close to the class II MHC *RT1.B* locus with the *RT1*^{a} and *RT1*^{l} haplotypes being *cim*^{a}, and the *RT1*^{u} and *RT1*^{c} haplotypes being *cim*^{b}.

received 100 μl of targets plus 100 μl of medium. Plates were incubated at 37°C for 5 h, then 100 μl of supernatant was harvested from each well and counted by gamma scintillation. All samples were performed in triplicate. Specific lysis was calculated from the formula: 100× [(experimental counts − SR)/(total input counts − SR)].

Results

Intracellular Transport of RT1.A^a. By immunoprecipitating class I molecules from detergent lysates of ^{35}S-methionine pulse-labeled Con A lymphoblasts, we compared the kinetics of processing of the major rat class I molecule RT1.A^a in a *cim^a* strain, PVG.R19 (*RT1.A^{a}B^{a}D^{a}C^c*), and a *cim^b* strain, PVG.R8 (*A^{a}B^{u}D^{u}C^u*). Processing during intracellular transport is indicated by the increase in relative molecular mass of the class I heavy chain from ~46,000 to ~47,000 in the course of the chase phase, an event that is associated with the transition from high-mannose to complex-type oligosaccharides and the subsequent addition of sialic acids to these complex-type structures in the trans-Golgi (4, 9, 25). Fig. 1 *A* illustrates that processing of RT1.A^a in PVG.R8 cells is very slow compared with PVG.R19 cells. Slow processing is also seen in the *cim^b* recombinant PVG.R1 (*A^{a}B^{c}D^{c}C^c*) but not in the *cim^a* strains PVG-*RT1^a* and DA (both *A^{a}B^{a}D^{a}C^{av1}*) (16; and S.J. Powis and G.W. Butcher, unpublished observa-

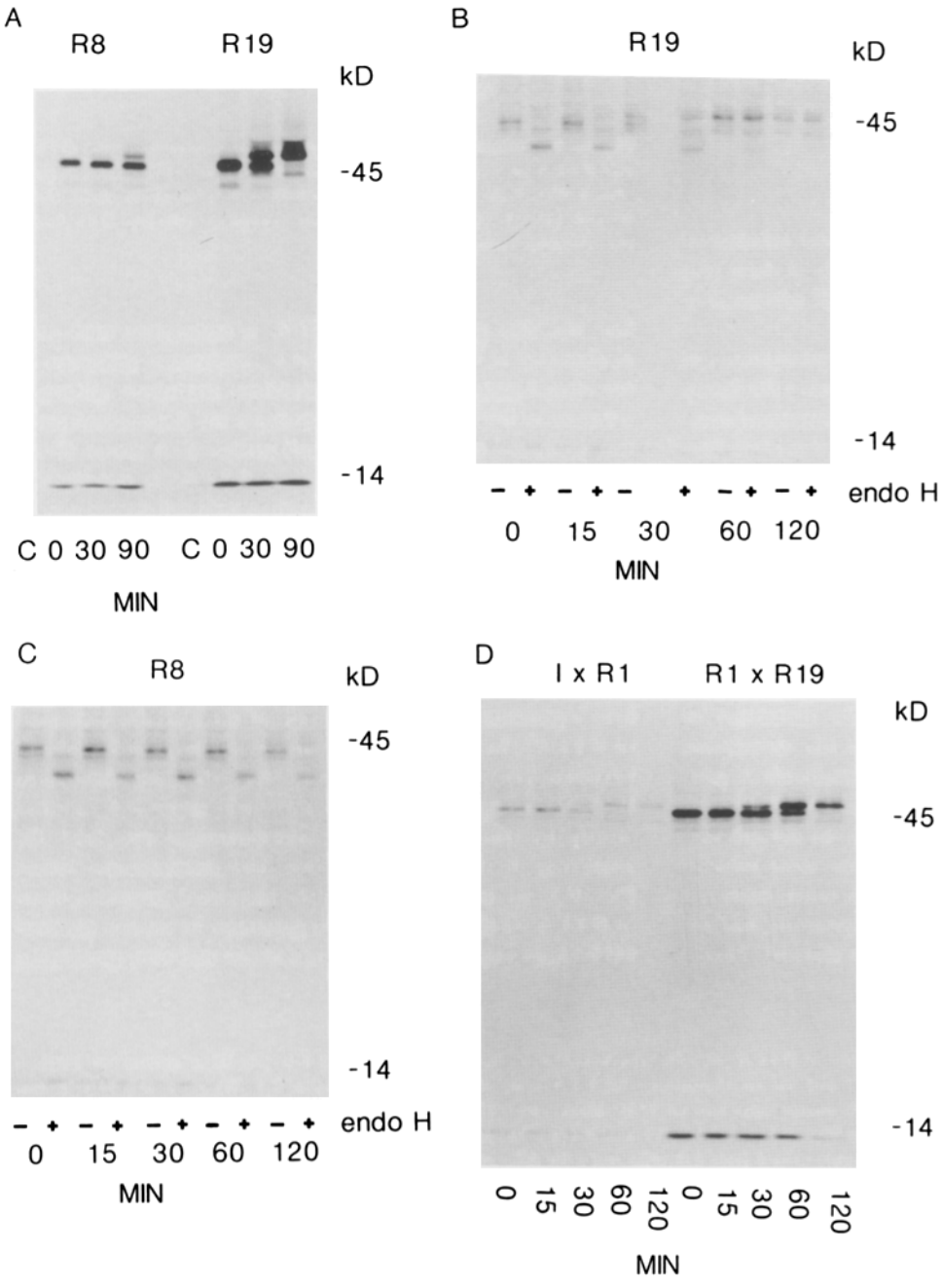


Figure 1. Pulse chase analysis of RT1.A^a immunoprecipitated from MHC recombinant rat Con A blasts. (*A*) PVG.R19 cells show an increase in the relative molecular mass of the heavy chain (46,000) beginning before 30 min of chase and being complete at 90 min of chase. PVG.R8 cells show only partial processing to the higher relative molecular mass form at 90 min of chase. (*B* and *C*) Pulse chase immunoprecipitate samples were treated with (+) or without (−) endo H. PVG.R19 cells process the class I heavy chain to an endo H–resistant form beginning ~15 min of chase (*B*). PVG.R8 cells retain endo H–sensitive heavy chains for >120 min of chase (*C*). (*D*) (PVG-*RT1^l* × PVG.R1)F$_1$ cells (l × R1) and (PVG.R1 × PVG.R19)F$_1$ cells (R1 × R19) show normal transport kinetics, demonstrating *trans*-action of *cim^a* carried by the *RT1^l* and *r19* haplotypes. (*B* and *C*) The endo H–resistant band immediately below the class I heavy chain represents a frequent contaminant band in internal labeling experiments. This band comigrates with actin purified from rat platelets (data not shown).

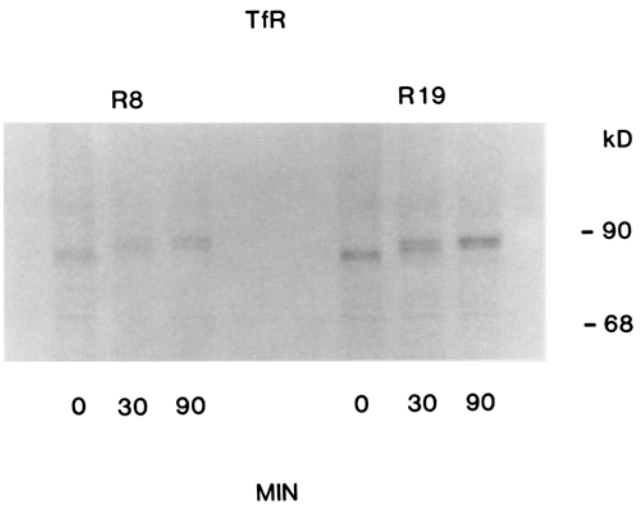


Figure 2. Pulse chase analysis of the TfR. PVG.R8 and PVG.R19 Con A blasts display identical, rapid transport kinetics for the TfR doublet as immunoprecipitated by MRC OX-26.

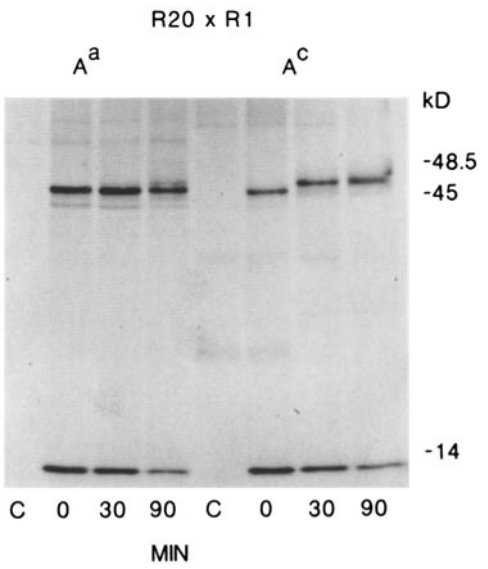


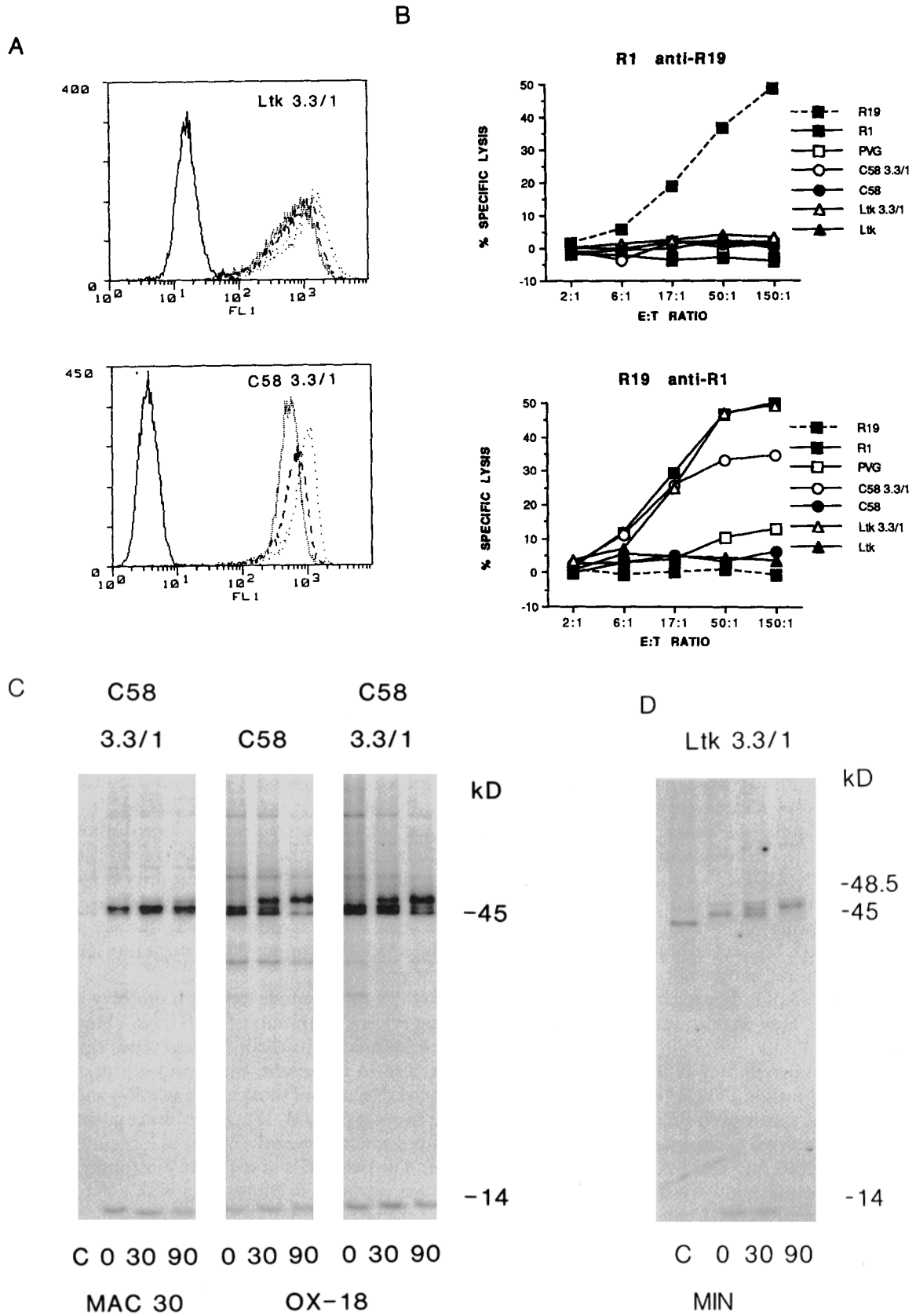
Figure 3. Allele specificity of RT1.A^a retention caused by *cim*^{b}. RT1.A^a and RT1.A^c were sequentially immunoprecipitated from lysates of pulse chased (PVG.R20 × PVG.R1)F_1 cells (R20 × R1), using the mAbs MAC 30 and YR5/12. RT1.A^a displays slow transport, whereas RT1.A^c displays normal transport kinetics.

tions). Note that all immunoprecipitates contain substantial quantities of β_2m, indicating that the assembly of these two components is either complete, or at least well advanced in the *cim*^{b} strains.

Endo H treatment of pulse chase immunoprecipitates of RT1.A^a demonstrates that in PVG.R19 cells (*cim*^{a}) the heavy chain acquires complex-type structures (endo H resistant) coincident with the increase in relative molecular mass but that in PVG.R8 cells (*cim*^{b}) the heavy chain retains high-mannose glycans (endo H sensitive) for an increased length of time (Fig. 1, *B* and *C*). PVG.R1 cells (*cim*^{b}) show the same pattern of endo H sensitivity as PVG.R8 cells (data not shown). This suggests that in PVG.R1 and PVG.R8 cells the RT1.A^a antigen is not transported from an early compartment such as the ER or *cis*-Golgi with the same kinetics as that observed for PVG.R19 cells. The lack of processing of the class I heavy chain is not due to a global cellular deficiency in glycosylation since pulse chase analysis of the transferrin receptor (TfR) shows that the M_r 90,000 molecule is processed at identical rates in both PVG.R8 and PVG.R19 cells (Fig. 2). Furthermore, the RT1.A^a molecules on the cell surface of PVG.R1 and PVG.R8 cells are of the same relative molecular mass as those from PVG.R19 cells when analyzed by cell surface iodination and immunoprecipitation, and are not sensitive to digestion by endo H (data not shown). Therefore, the slow processing of RT1.A^a in association with *cim*^{b}, as depicted in Fig. 1, *A*, *B*, and *C*, represents retention of the class I antigen during intracellular transport, although a transit of RT1.A^a molecules through the maturation pathway sufficient to populate the cell surface does occur. However, this kinetic effect of *cim* on RT1.A^a is presumably responsible for the previously noted reduced expression of RT1.A^a by PVG.R1 cells (26).

Trans-acting Rescue of RT1.A^a Transport. The *cim*^{a} and *cim*^{b} alleles were originally defined by their controlling effect on the alloantigenic specificity of RT1.A^a as defined by CTLs, *cim*^{a} and *cim*^{b} determining the A^{a+} and A^{a-} forms respectively (16). The above results suggest that these alleles also have a profound effect on the rate of intracellular transport of RT1.A^a. In our previous study we showed in F_1 hybrids between *cim*^{a} and *cim*^{b} strains that the *cim*^{a}-dependent RT1.A^{a+} antigenic phenotype alone was expressed; in other words, by this criterion, *cim*^{a} was dominant over *cim*^{b}. By analyzing F_1 hybrids with the *cim*^{b} recombinant strain PVG.R1, we were able to use the expressed antigenic form of RT1.A^a to determine the *cim* genotype of various parental MHC haplotypes. The correlation of fast and slow processing

Figure 4. Antigenic and biosynthetic characteristics of RT1.A^a in cells transfected with the RT1.A^a cDNA 3.3/1. (*A*) Flow cytometric analysis of the mouse fibroblast L cell transfectant Ltk-3.3/1 and the rat thymoma C58-3.3/1 using the anti-RT1.A^a mAbs R2/15S (. . .), R3/13 (...), and MAC 30 (- - -). The solid line indicates staining with second stage anti-rat FITC alone. (*B*) CTL analysis of the Ltk-3.3/1 and C58-3.3/1 transfectant cell lines. PVG.R19 anti-PVG.R1 (R19 anti-R1) and PVG.R1 anti-R19 (R1 anti-R19) CTL were tested for their ability to lyse the transfected cell lines Ltk-3.3/1 (Δ), C58-3.3/1 (○), the untransfected control lines Ltk (▲) and C58 (●), and the rat con A blasts PVG (□), PVG.R1 (■), and PVG.R19 (-■-). Lysis of the transfected cell lines by R19 anti-R1 (anti-A^{a-}) but not R1 anti-R19 (anti-A^{a+}) CTL indicates expression of the A^{a-} alloantigenic form of RT1.A^a. (*C*) Pulse chase analysis of C58-3.3/1 transfectants and untransfected C58 cell lines. RT1.A^a was immunoprecipitated from C58-3.3/1 cells using MAC 30-Sepharose (*left*), and exhibits slow transport. MRC OX-18-Sepharose was then used on the same lysate to isolate *RT1*^{u} class I molecules, which show normal transport (*right*) similar to the MRC OX-18-Sepharose signal isolated from untransfected C58 cells (*center*).



(*D*) Pulse chase analysis of Ltk-3.3/1 cells. RT1.A^a was immunoprecipitated from pulse-labeled Ltk-3.3/1 cells using MAC 30-Sepharose. The control track (C) indicates presumed actin contamination. RT1.A^a is processed with normal kinetics in the mouse cells despite displaying the A^{a-} alloantigenic form at the cell surface.

rates of RT1.A^a with the expression of A^{a+} and A^{a-}, respectively, prompted us to examine the transport of RT1.A^a in cells from F_1 hybrids between the PVG.R1 strain and PVG-*RT1^l*(LEW), a strain carrying the dominant *cim^a* allele in the *RT1^l* haplotype. In Fig. 1 *D*, where the MAC 30 (noncross-reactive on RT1.A^l) has been used to immunoprecipitate the RT1.A^a molecule from (PVG-RT1^l[LEW] × PVG.R1)F_1 cells, it can be seen that RT1.A^a is transported at a rate similar to that observed for PVG.R19 cells (see Fig. 1 *A*). Therefore, through *trans*-action of the dominant *cim^a* allele carried by the *RT1^l* haplotype, normal transport kinetics are returned to the RT1.A^a class I antigen. Similarly, in (PVG.R1 × PVG.R19)F_1 cells, normal transport of the class I antigen occurs, with little if any of the heavy chain being retained in the low relative molecular mass form (Fig. 1 *D*).

Retention of RT1.A Is Class I Allele Specific. As described above, in conjunction with the *cim^b* allele, RT1.A^a is subject to retention within the ER or cis-Golgi. We next asked whether another allotype of RT1.A would also be subject to retention in the presence of *cim^b*. Pulse chase analysis was therefore performed on cells from an F_1 hybrid expressing two different RT1.A allotypes in a homozygous *cim^b* context. (PVG.R20 × PVG.R1)F_1 cells (*cim^b/cim^b*, *A^{c}B^{c}D^{c}C^{av1}* × *A^{a}B^{c}D^{c}C^c*), which express A^{a-} as determined by CTL analysis (16a), were chosen for this experiment. RT1.A^a and RT1.A^c were immunoprecipitated sequentially from the same lysate using the RT1.A^a-specific mAb MAC 30 and the RT1.A^c-specific mAb YR5/12. As shown in Fig. 3, RT1.A^a was subject to the *cim^b*-dependent retention, whereas RT1.A^c was transported with typical rapid kinetics. Similar results have been obtained for MHC heterozygous cells bearing the RT1.A^n allotype. Thus, the *cim^b*-dependent retention phenomenon is specific for the RT1.A^a allelic product.

Transport and Antigenicity of RT1.A^a in Transfected Cell Lines. With the aim of cloning *cim* by complementation in a transfection system, we have introduced the recently derived RT1.A^a cDNA 3.3/1 (23) into several in vitro cell lines. Data are presented here for one rat transfectant cell line, C58-3.3/1, the host cell being *RT1^u* (*cim^b*); and for one mouse transfectant, Ltk-3.3/1. Fig. 4 *A* indicates the expression of 3.3/1 product on the cell surface of the transfectant cell lines Ltk-3.3/1 and C58-3.3/1, detected by flow cytometry using three mAbs to two distinct epitopes on the RT1.A^a antigen (19).

Utilizing the ability to raise effector CTL populations directed at A^{a+} in the combination PVG.R1 anti-R19, and against A^{a-} in the reciprocal system PVG.R19 anti-PVG.R1 (16a) the transfectants were analyzed to determine which form of RT1.A^a they expressed. PVG.R19 anti-PVG.R1 effectors (anti-A^{a-}) killed both the rat and mouse transfected cell lines along with the positive control PVG.R1 targets (Fig. 4 *B*). Neither of the untransfected cell lines were killed, nor were the control targets PVG (*RT1^c*) or PVG.R19. In the reciprocal CTL combination, PVG.R1 anti-PVG.R19 effector cells (anti-A^{a+}) killed only the positive control PVG.R19 targets, and neither of the transfectants. Thus, the alloantigenic status of RT1.A^a when expressed in the mouse *H-2^k* haplotype L cells and the rat *RT1^u* haplotype C58 cell line is A^{a-}. The same result has also been obtained for RT1.A^a transfectants of the rat *RT1^u* haplotype Y3 cell line, and the mouse BW5147 cell line (*H-2^k*) (data not shown). mAb blocking studies indicate that the CTL are directed at the RT1.A^a antigen on the transfectants (data not shown). It is important to note here that the 3.3/1 cDNA was obtained from the *cim^a*, A^{a+}-expressing DA rat strain. Thus, this class I gene can be expressed in the two antigenic forms A^{a+} and A^{a-} depending on the host cell genotype.

Pulse chase analysis of the C58-3.3/1 line showed slow transport of RT1.A^a while the endogenous RT1^u class I molecules were transported rapidly (Fig. 4 *C*). The differential transport kinetics of the two class I allelic products in the same *cim^b* rat transfectant cell was thus consistent with the behavior of RT1.A^a and RT1.A^c in the *cim^b* (PVG.R20 × PVG.R1)F_1 hybrid cells described in the previous section. Furthermore, in the rat transfectant cells, the typical *cim^b*-dependent slow transport kinetics were associated, as expected, with the *cim^b*-dependent RT1.A^{a-} antigenic phenotype. However, when the same experiment was performed on Ltk-3.3/1 cells, we were surprised to observe a rapid rate of transport for RT1.A^a (Fig. 4 *D*), which, for the first time, separated the A^{a-} alloantigenic phenotype from the "slow kinetics" phenotype. Similar results were obtained in the previously described mouse BW 3.3/1 cell line (data not shown), indicating that this result is not due to the nonlymphoid origin of the Ltk cell line.

Discussion

MHC class I and class II molecules are cell surface glycoproteins that present antigens to T lymphocytes bearing specific receptors. Presentation is presumably achieved by the peptide antigen binding noncovalently to a groove formed between two α-helices on the upper surface of the class I molecule (5). It now seems likely that the majority of MHC class I molecules acquire their peptide antigens in the endoplasmic reticulum soon after biosynthesis (6, 8), thereby providing a means by which the immune system can screen for intracellular pathogens.

The mechanism of peptide loading is probably one cause of the long evident complexity of MHC class I biosynthesis. Recent evidence suggests that, over and above the requirement for β_2m to be present, both antigenic peptides and MHC-encoded factors are necessary for assembly and efficient transport to occur (4, 7, 11, 12). The evidence presented here details important characteristics of the *cim* locus in the MHC of the rat. The two alleles of *cim* exert profoundly different effects when expressed in conjunction with a major class I antigen of the rat, RT1.A^a. The dominant allele *cim^a* allows normal intracellular transport of RT1.A^a, and determines expression of the A^{a+} alloantigenic form of the molecule at the cell surface. The recessive *cim^b* allele, when homozygous, is associated with slow RT1.A^a transport and with the expression of the A^{a-} alloantigenic form. That the *cim* gene can act in *trans* is demonstrated by the rescue of rapid transport kinetics for RT1.A^a by the *cim^a* allele carried by the

RT1^{l} haplotype (Fig. 1 *D*) (27) combined with expression of the A^{a+} alloantigenic form.

The inefficient transport of RT1.A^{a} caused by *cim*^{b} is also class I allele specific. F_1 cells that are *cim*^{b} homozygous and express both RT1.A^{a} and RT1.A^{c} only display retention of the RT1.A^{a} molecule (Fig. 3). This class I allele specificity is also demonstrated in a rat cell line of the *RT1*^{u} haplotype that expresses RT1.A^{a} due to a transfected cDNA (Fig. 4 *C*).

What clues does the abnormal phenotype of A^{a-}, *cim*^{b} cells such as PVG.R1 and PVG.R8 provide as to the function of the *cim* gene product in class I MHC assembly? Two features of our data are informative:

cim Activity Is Sensitive to Polymorphic Residues in RT1.A

Since most class I polymorphism is invested in the α1-α2 domains (28), it is likely that this portion of the molecule is responsible for the class I allele dependency of *cim*. We envisage two general schemes compatible with present and previous (16) data whereby α1-α2 polymorphism might impinge on *cim* activity.

Scheme A. The *cim* gene product binds directly to the class I molecule at a site containing allele-specific amino acid residues. The *cim*^{a} and *cim*^{b} products differ with respect to this interaction. As a result of this binding, the allelic *cim* products are in a position to influence differentially the nature of the peptides that are bound in the "peptide-binding groove". This influence could either be indirect, via tertiary structural modification of the groove, or direct, via interaction of the *cim* product with peptides as they try to enter the molecular assembly. If successful entry of peptide is followed by release of the bound class I molecule from the *cim* product, then the latter could engage in multiple rounds of binding and release. Retention of RT1.A^{a} for unusually long periods in the ER of PVG.R1 or PVG.R8 cells could result from abnormal interaction of the *cim*^{b} product with the RT.1A^{a} heavy chain, e.g., a long dissociation time brought about either by an excessively high binding energy or disruption of the mechanism of peptide entry and *cim* release.

Scheme B. The *cim* product makes peptides available for MHC class I assembly without itself interacting directly with the class I molecule. The products of the two *cim* alleles deliver two different sets of endogenous peptides (p) to the assembling class I heavy chains (H) and β_2m (L). The class I allele specificity could result if, for instance, the spectrum of peptides provided by *cim*^{b} was physicochemically ill-suited to the peptide binding site of RT1.A^{a} while being appropriate to RT1.A^{u}, RT1.A^{n}, RT1.A^{c}, etc. In the presence of *cim*^{b} (homozygous), the delay in RT1.A^{a} biosynthesis would result from relative peptide starvation, and transit out of the ER would be delayed if complete (HLp) molecules were favored for exit.

Scheme A is compatible with the notion that the *cim* product fulfills a "chaperonin"-like function in class I assembly (29), while the view of *cim* in scheme B is consistent with either the peptide transporter hypothesized by Townsend et al. (30), modified to include selective transport properties, or with allelically determined endopeptidases of distinct sequence specificity engaged in the provision of peptides.

Association of the MHC Class I Heavy Chain and β_2m to Form an HL Complex Occurs Readily in the Aberrant Combination of cim^{b} *and RT1.A*^{a} *Found in the PVG.R1 and PVG.R8 Rat Strains*

All of our immunoprecipitates of A^{a-} contain substantial quantities of β_2m, irrespective of the specific mAb used, suggesting that the "defect" in these cases is at a stage subsequent to association of H and L. This would imply a reaction sequence: (*a*) H + L $\rightleftarrows$ HL; (*b*) HL + p $\rightleftarrows$ HLp; the latter step involving the *cim* product. As Townsend et al. (7) have pointed out, however, several potential reaction sequences may be available during MHC class I assembly and the pathways used may be influenced by the availability of the reactants. Scarcity of peptide may therefore result in association and accumulation of HL (without p) by default, even though the reaction H + p $\rightleftarrows$ Hp might be the predominant first step in "normal" circumstances. It is therefore premature to assume that *cim* cannot interact, directly or indirectly, with the free H chain, despite the phenotype of PVG.R1 and PVG.R8.

In those cell lines of human and murine origin in which defective class I assembly has recently been studied (11, 12, 30), it seems clear that the defect(s) involved severely impairs the transport of class I molecules and their ability to act as antigen-presenting molecules. Although no mapping data are available for the defect in the murine RMA-S cell line, the class II linkage of the defect in the human .174 cell line is compatible with our mapping of *cim*, which appears to lie between *RT1.Hα* (*DPα*-like) and RT1.Bα (DQα-like) (16a). If we consider for the time being that the RMA-S, .174, and *cim* phenomena are due to the action of a single gene system, then the mutant cell lines can perhaps be classified as *cim*^{null}, while the *cim*^{b}, RT1.A^{a} combination should be considered as intermediate between *cim*^{null} and an optimal wild-type condition, since it is clear that A^{a-} can act as a restriction element (Fig. 4 *B*) (16). It is interesting to speculate that there may be other *cim*-related genes mapping within the MHC. Indeed, the antigenicity of Qa-1 in the mouse is under the influence of an allelic genetic system with features resembling *cim*, but mapping to the *H-2D* region (13), and recent observations on the expression of *HLA-B27* in transgenic mice show *H-2D* region control of expression (14).

An interesting feature apparent when comparing the *cim*/*RT1.A*^{a} system in rats and the *Qdm*/*Qa-1* system in mice (13) is that in both cases the modifying gene maps relatively close to the locus for the class I antigen it serves. This raises the possibility that natural selection may favor certain *cis* allelic combinations of *RT1.A* and *cim*. The biosynthetic inefficiency of the *RT1.A*^{a}, *cim*^{b} combination found in the laboratory recombinant haplotypes *r1* and *r8* compared with *RT1.A*^{a}, *cim*^{a} in the parental *a* haplotype may be sufficient for selection to operate, and recalls the inferiority of some *trans* cf *cis* combinations of MHC class II α and β chains uncovered by Germain et al. (31).

The final area of experimentation that warrants discussion here is the expression of MHC class I genes or cDNAs in host cells of different species. In an intriguing series of experiments, Alexander and associates (12, 33, 34) studied the expression of mouse class I molecules after transfection of human mutant cell lines (.174 and T2). Whereas these cells were defective in the expression of endogenous or transfected HLA class I molecules, they could express mouse class I molecules at "normal" levels. Subsequent analysis, however, has indicated that the mouse molecules that reach the surface of T2 are aberrant in that they fail to present endogenous peptides, and like the small numbers of HLA class I molecules that are detectable on the cell, are apparently "empty" (peptide-free) molecules (12, 34). Thus, the cim^{null} genotype of T2 consistently determines failure to present peptide, but does not correlate fully with transport kinetics or cell surface expression of MHC class I. This description shows close parallels with the results of *trans*-species transfections reported here (Fig. 4). When the rat RT1.A^a cDNA 3.3/1 was expressed in mouse L cells, the CTL-defined A^{a-} phenotype (normally associated with cim^b) was found, unexpectedly, to be associated with rapid transport of RT1.A^a. So, the *cim* genotype correlates better with putative peptide loading of MHC class I than with transport kinetics. Therefore, in agreement with Alexander et al. (32), we find it conceptually attractive to separate the peptide delivery and/or loading process controlled by *cim* (which we take to be the same gene as that mutant in .174 and its derivatives, and possibly in RMA-S) from another, species-specific, component that influences transport kinetics and may act to retain in the ER class I molecules that have not yet been loaded with peptide.

Experiments aimed at the identification of the *cim* gene product are in progress. Recently, cDNAs derived from two closely linked genes with homology to multi-drug resistance genes and to bacterial hemolysin transport proteins (ATP-binding cassette family of membrane transporter protein), whose genetic mappings are consistent with the known location of *cim*, have been isolated from a DA ($RT1^a$, cim^a) Con A blast library (17). If these cDNAs do represent the *cim* gene, then this obviously provides support for the idea of a peptide transporter with selective properties.

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Address correspondence to Geoffrey W. Butcher, Department of Immunology, Institute of Animal Physiology & Genetics Research, Cambridge Research Station, Babraham, Cambridge CB2 4AT, UK.

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