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

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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⁺, 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⁺ within the endoplasmic reticulum or cis-Golgi. In appropriate F₁ hybrid cells heterozygous for RT1.A⁺ and another class I MHC allele, RT1.A°, only the RT1.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 Mr 45,000 transmembrane heavy chain noncovalently associated with the Mr 12,000 β₂-microglobulin (β₂m)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 β₂m 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, β₂m, 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⁺B⁺D⁺C⁺) and PVG.R8 (A⁻ B⁻D⁻C⁻) recombinant rats resulted in the altered antigenicity of the RT1.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⁺ molecule, namely A⁺⁺ and A⁺⁻, in the presence of the cim⁺ (dominant) and cim⁻ (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⁺ both in lymphocytes from the recombinant rat strains and also in rat and mouse cell lines transfected with a cDNA encoding RT1.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% CO₂ in air.

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

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tions, while MAC 30 (IgG2c) was derived from a PVG-RT1* anti-PVG, R5 immunization. R3/13 recognizes the P site, and R2/15S and MAC 30 recognize the S site of RT1.A* (19). The anti-RT1.A* mAb YR5/12 (IgG2b) was obtained from an AO anti-PVG (RT1* anti-RT1*) 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* 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-HGPR/RT. Cells were selected in medium containing hypoxanthine, aminopterin, and thymidine (HAT), and sorted for expression of RT1.A* by flow cytometry.

Pulse Chase Labeling. Con A lymphoblasts, generated by incubation of lymph node cells for 48 h at 37°C, were dispersed into 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 I[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 MgCl2, 1 mM PMSF, 20 mM Tris-HCl, pH 8.0). After a 30-min incubation on ice, the lysates were spun at 11,000 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 preclared for 1 h at 4°C with 20 μl (packed volume) of Sepharose coupled with an irrelevant mAb. RT1.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* signal, 20 μl of MRC OX-18-Sepharose was added or, for RT1.A*, 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, pH 8.0) and boiled for 2 min in 20 μl of SDS sample buffer (2% [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 preclared 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 10% 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 6 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* and A* forms of RT1.A* were generated in MLC using cells from primed animals. PVG.R1 and PVG.R19 animals were primed against A* and A* forms, 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 (105 cells 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 -3 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 51Cr-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 6 cells/ml, and serial threefold dilutions prepared. 100 μl of effector cells plus 100 μl of labeled targets at 10 6 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 | cim allele* |
| Independent inbred | c | c | c | c | b |
| PVG             | r1 | a | c | c | c | b |
| MHC congenic    | r8 | a | u | u | b |
| PVG-RT1(LEW)    | r19 | a | a | a | c | a |
| PVG.R1          | r20 | c | c | c | av1 | b |

* cim maps close to the class II MHC RT1.B locus with the RT1* and RT1* haplotypes being cim, and the RT1* and RT1* haplotypes being cim.
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 × \( \frac{(\text{experimental counts} - \text{SR})}{(\text{total input counts} - \text{SR})} \).

Results

Intracellular Transport of RT1.Aa. By immunoprecipitating class I molecules from detergent lysates of 35S-methionine pulse-labeled Con A lymphoblasts, we compared the kinetics of processing of the major rat class I molecule RT1.Aa in a cim° strain, PVG.R19 (RT1.A°B°D°C°), and a cim° strain, PVG.R8 (A°B°D°C°). 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 illustrates that processing of RT1.Aa in PVG.R8 cells is very slow compared with PVG.R19 cells. Slow processing is also seen in the cim° recombinant PVG.R1 (A°B°D°C°) but not in the cim° strains PVG-RTI° and DA (both A°B°D°C°°°) (16; and S.J. Powis and G.W. Butcher, unpublished observa-

Figure 1. Pulse chase analysis of RT1.Aa 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) Cells were treated with (+) or without (–) endo H. PVG.R19 cells process the class I heavy chain to a 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-RTI° x PVG.R1)F1 cells (I x R1) and (PVG.R1 x PVG.R19)F1 cells (R1 x R19) show normal transport kinetics, demonstrating trans-action of cim° carried by the RT1° and R1° 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.

Note that all immunoprecipitates contain substantial quantities of β2m, indicating that the assembly of these two components is either complete, or at least well advanced in the cim" strains.

Endo H treatment of pulse chase immunoprecipitates of RTLAa demonstrates that in PVG.R19 cells (cim") 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") the heavy chain retains high-mannose glycans (endo H sensitive) for an increased length of time (Fig. 1, B and C). PVG.R1 cells (cimb) 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 RTLAa 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 due to a global cellular deficiency in glycosylation since pulse chase analysis of the transferrin receptor (TfR) shows that the Mr 90,000 molecule is processed at identical rates in both PVG.R8 and PVG.R19 cells (Fig. 2). Furthermore, the RTLAa 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 RTLAa in association with cim", as depicted in Fig. 1, A, B, and C, represents retention of the class I antigen during intracellular transport, although a transit of RTLAa molecules through the maturation pathway sufficient to populate the cell surface does occur. However, this kinetic effect of cim on RTLAa is presumably responsible for the previously noted reduced expression of RTLAa by PVG.R1 cells (26).

Trans-acting Rescue of RT1.Aa Transport. The cim" and cim" alleles were originally defined by their controlling effect on the alloantigenic specificity of RTLAa as defined by CTLs, cim" and cim" determining the A"+ and A"- forms respectively (16). The above results suggest that these alleles also have a profound effect on the rate of intracellular transport of RTLAa. In our previous study we showed in F1 hybrids between cim" and cim" strains that the cim"-dependent RTLAa-" antigenic phenotype alone was expressed; in other words, by this criterion, cim" was dominant over cim". By analyzing F1 hybrids with the cim" recombinant strain PVG.R1, we were able to use the expressed antigenic form of RTLAa to determine the cim genotype of various parental MHc haplotypes. The correlation of fast and slow processing
(D) Pulse chase analysis of Ltk-3.3/1 cells. RT1.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* is processed with normal kinetics in the mouse cells despite displaying the A* alloantigenic form at the cell surface.
rates of RT1.A with the expression of A⁺⁺ and A⁺⁻, respectively, prompted us to examine the transport of RT1.A in cells from F₁ hybrids between the PVG.R1 strain and PVG-RT1(LEW), a strain carrying the dominant cim⁺ allele in the RT1⁺ haplotype. In Fig. 1 D, where the MAC 30 (noncross-reactive on RT1.A) has been used to immunoprecipitate the RT1.A molecule from (PVG-RT1(LEW) × PVG.R1)F₁ cells, it can be seen that RT1.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⁺ allele carried by the RT1⁺ haplotype, normal transport kinetics are returned to the RT1.A⁺ class I antigen. Similarly, in (PVG.R1 × PVG.R19)F₁ 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⁺ allele, RT1.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⁺. Pulse chase analysis was therefore performed on cells from an F₁ hybrid expressing two different RT1.A allotypes in a homozygous cim⁺ context. (PVG.R20 × PVG.R19)F₁ cells (cim⁺/cim⁺, A⁺B⁺D⁺C⁺ × A⁺B⁺D⁺C⁺), which express A⁺⁺ as determined by CTL analysis (16a), were chosen for this experiment. RT1.A⁺ and RT1.A⁻ were immunoprecipitated sequentially from the same lysate using the RT1.A⁺-specific mAb MAC 30 and the RT1.A⁻-specific mAb YR5/12. As shown in Fig. 3, RT1.A⁺ was subject to the cim⁺-dependent retention, whereas RT1.A⁻ was transported with typical rapid kinetics. Similar results have been obtained for MHC heterozygous cells bearing the RT1.A° allotype. Thus, the cim⁺-dependent retention phenomenon is specific for the RT1.A⁺ allelic product.

Transport and Antigenicity of RT1.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⁺ cDNA 3.3/1 (23) into several in vitro cell lines. Data are presented here for one rat transfected cell line, C58-3.3/1, the host cell being RT1⁺ (cim⁺); and for one mouse transfected, Ltk⁻3.3/1. Fig. 4 A indicates the expression of 3.3/1 product on the cell surface of the transfected 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⁺ antigen (19).

Utilizing the ability to raise effector CTL populations directed at A⁺⁺ in the combination PVG.R1 anti-R.19, and against A⁺⁻ in the reciprocal system PVG.R19 anti-PVG.R1 (16a) the transfecteds were analyzed to determine which form of RT1.A they expressed. PVG.R19 anti-PVG.R1 effectors (anti-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⁺) or PVG.R19. In the reciprocal CTL combination, PVG.R1 anti-PVG.R19 effector cells (anti-A⁺⁺) killed only the positive control PVG.R19 targets, and neither of the transfecteds. Thus, the alloantigenic status of RT1.A⁺ when expressed in the mouse H-2k haplotype L cells and the rat RT1⁺ haplotype C58 cell line is A⁺⁻. The same result has also been obtained for RT1.A⁺ transfectants of the rat RT1⁺ haplotype Y3 cell line, and the mouse BW5147 cell line (H-2k) (data not shown). mAb blocking studies indicate that the CTL are directed at the RT1.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⁺⁻ expressing DA rat strain. Thus, this class I gene can be expressed in the two antigenic forms A⁺⁺ and A⁺⁻ depending on the host cell genotype.

Pulse chase analysis of the C58-3.3/1 line showed slow transport of RT1.A⁺ while the endogenous RT1⁺ molecules were transported rapidly (Fig. 4 C). The differential transport kinetics of the two class I allelic products in the same cim⁺ rat transfected cell was thus consistent with the behavior of RT1.A⁺ and RT1.A⁻ in the cim⁺ (PVG.R20 × PVG.R19)F₁ hybrids described in the previous section. Furthermore, in the rat transfected cells, the typical cim⁺-dependent slow transport kinetics were associated, as expected, with the cim⁺-dependent RT1.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⁺ (Fig. 4 D), which, for the first time, separated the 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 (9). 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 β₂m 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⁺. The dominant allele cim⁺ allows normal intracellular transport of RT1.A⁺, and determines expression of the A⁺⁺ alloantigenic form of the molecule at the cell surface. The recessive cim⁻ allele, when homozygous, is associated with slow RT1.A⁺ transport and with the expression of the A⁺⁻ alloantigenic form. That the cim gene can act in trans is demonstrated by the rescue of rapid transport kinetics for RT1.A⁺ by the cim⁺ allele carried by the
RT1 haplotype (Fig. 1 D) (27) combined with expression of the Aq alloantigenic form.

The inefficient transport of RT1.Aq caused by cimb is also class I allele specific. Fj cells that are cimb homozygous and express both RT1.Aq and RT1.Aq only display retention of the RT1.Aq molecule (Fig. 3). This class I allele specificity is also demonstrated in a rat cell line of the RT1 haplotype that expresses RT1.Aq due to a transfected cDNA (Fig. 4 C).

What clues does the abnormal phenotype of Aq, cim 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 cimb and cimb 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.Aq for unusually long periods in the ER of PVG.R1 or PVG.R8 cells could result from abnormal interaction of the cimb product with the RT1.Aq 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 β2m (L). The class I allele specificity could result if, for instance, the spectrum of peptides provided by cimb was physicochemically ill-suited to the peptide binding site of RT1.Aq while being appropriate to RT1.Aq, RT1.Aq, RT1.Aq, etc. In the presence of cimb (homozygous), the delay in RT1.Aq 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 β2m to Form an HL Complex Occurs Readily in the Aberrant Combination of cim and RT1.Aq Found in the PVG.R1 and PVG.R8 Rat Strains

All of our immunoprecipitates of Aq contain substantial quantities of β2m, 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 → HL; (b) HL + p → 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 → 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 cimnull, while the cimb, RT1.Aq combination should be considered as intermediate between cimnull and an optimal wild-type condition, since it is clear that Aq 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.Aq 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.Aq, cimb combination found in the laboratory recombinant haplotypes r1 and r8 compared with RT1.Aq, cimb in the parental a haplotype may be sufficient for selection to operate, and recalls the inferiority of some trans of 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<sup>null</sup> 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<sup>+</sup> cDNA 3.3/1 was expressed in mouse L cells, the CTL-defined A<sup>+</sup>-phenotype (normally associated with cim<sup>+</sup>) was found, unexpectedly, to be associated with rapid transport of RT1.A<sup>+</sup>. 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<sup>+</sup>, cim<sup>+</sup>) 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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