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19. Twelve (nine male and three female) strongly right-handed subjects (age range, 23 to 36 years; mean, 27.6 years) who had not participated in the object study were tested. Informed consent was obtained in writing. The written names of the objects used in the first experiment were presented. Stimulus presentation parameters were identical to those used in the first experiment. Mean ± SEM for voice response time = 901 ± 29.2 ms for generating color words and 886 ± 26.6 ms for generating action words ($P > 0.10$).
20. Findings from studies of patients with focal brain lesions argue against alternative interpretations of these activations as the sites for storage of the color and action words themselves, or of word-specific retrieval mechanisms located at sites distant from those where information is stored. A patient with a color anomia can produce color words. What the patient cannot do is correctly name the colors seen or correctly answer questions about object-associated colors (the patient will respond with an inappropriate color word). To show areas of activity by functional brain imaging technologies, it is necessary for subjects to be engaged in performing a task. Thus, it might be formally more accurate to state that the activations in the temporal lobes show the sites of storage during the act of retrieval. In this sense, however, storage and retrieval cannot be distinguished.
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25. An important question that remains to be resolved concerns the role of mental imagery in eliciting the activations associated with the attribute word generation tasks. Most subjects reported color and motion imagery while generating color words and action words, respectively. It has been proposed that mental imagery is dependent on the top-down influence of brain areas that contain stored representations on lower-level visual-processing areas in the occipital lobe (*27*). Evidence that the occipital cortex may serve as the representational medium for visual images has been obtained from studies of patients with focal lesions [M. J. Farah, M. J. Soso, R. M. Dasheiff, *J. Exp. Psychol. Hum. Percept. Perform.* **18**, 241 (1992)] and from PET studies of normal individuals (*27*). Our failure to observe activity in the occipital cortex during the word generation tasks could have resulted from the involvement of mental imagery or lower-level visual processing (or both) in the baseline tasks. The regions identified in our study might also be activated, perhaps in concert with the occipital cortex, if subjects were explicitly instructed to imagine objects in a particular color and to imagine objects being used. If so, our findings would suggest that different components of an image, such as color and motion, would depend on the activation of representations of object color stored in the ventral temporal lobe and representations of object action stored in the middle temporal gyrus.
26. Neither frontal nor parietal regions involved in object manipulation and visual guidance of reaching and grasping were selectively activated when subjects produced action words. Failure to activate these regions may reflect the fact that many of the objects used in the studies would not normally be experienced by reaching, grasping, or manipulation. Examples (followed by the most common response) include wheel (roll), cannon (shoot), helicopter (fly), telescope (see), gate (open), bed (sleep), and chair (sit). Objects were not limited to small, manipulable items such as tools because these items invariably elicit "silver" or "gray" as color responses. Brain regions close to those that are active during the actual manipulation of objects may be activated if subjects generated action words only in response to small, manipulable objects.
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29. Additional regions of activity were located in the inferior frontal (+32, +22, +16), inferior parietal (+48, −46, +32), and parietal-occipital sulcus (+18, −70, +32) of the right hemisphere in the object study, and the left mid-frontal region (−24, +20, +40) in the word study.
30. Additional regions were located in the right cerebellum: (+18, −72, −28 and +4, −44, −24) in the object study and (+42, −76, −20) in the word study.
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Dependence of Peptide Binding by MHC Class I Molecules on Their Interaction with TAP

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Major histocompatibility complex (MHC) class I molecules bind peptides that are delivered from the cytosol into the endoplasmic reticulum by the MHC-encoded transporter associated with antigen processing (TAP). Peptide capture by immature heterodimers of class I heavy chains and β_2-microglobulin may be facilitated by their physical association with TAP. A genetic defect in a human mutant cell line causes the complete failure of diverse class I heterodimers to associate with TAP. This deficiency impairs the ability of the class I heterodimers to efficiently capture peptides and results from loss of function of an unidentified gene or genes linked to the MHC.

MHC class I molecules export peptides derived from cytosolic protein degradation to the cell surface and thus enable cytotoxic T cells to detect intracellular antigen (*1*). They consist of a membrane-anchored polymorphic class I heavy chain, soluble β_2-microglobulin (β_2M), and a peptide ligand of 8 to 10 amino acids with an allele-specific sequence motif (*2*). The complete subunit assembly of class I molecules is usually required for their conformational stability, maturation, and normal surface expression and involves accessory molecules (*2*). Upon entering the endoplasmic reticulum (ER), newly synthesized human class I heavy chains are retained by calnexin until they combine with β_2M (*3*). The immature class I heavy chain–β_2M heterodimers then associate with TAP transporters (*4*), which consist of the MHC-encoded TAP1 and TAP2 subunits and deliver the peptides that are mainly bound by class I heterodimers from the cytosol into the lumen of the ER (*5–7*). This physical interaction may be coupled to peptide binding by class I heterodimers, because dissociation from TAP correlates with their conversion into stably conformed class I molecules. Thus, by interacting with TAP, class I heterodimers may gain access to peptides before these may be diluted and possibly degraded in the lumen of the ER. However, it is unknown whether this proposed mechanism promotes peptide binding by class I heterodimers in living cells.

In defining the assembly of class I molecules, mutant cell lines with specific defects have been instrumental. In the human mutant lymphoblastoid cell line (LCL) 721.220, an unknown defect impairs the surface expression of class I molecules. These cells express functional β_2M and TAP, as indicated by transcomplementation of Daudi (β_2M$^-$) and mutant LCL 721.174 (TAP$^-$) cells after fusion (*8*). These cells have been isolated after repeated mutagenesis and selection against the surface class I molecules encoded in a hemizygous MHC, resulting in deletion of *HLA-A* and *-B* and in reduced surface levels of HLA-C (*9*). However, as with HLA-C, 220 cells are also unable to express normal surface amounts of several HLA-A and -B alleles after gene transfer–mediated reconstitution of biosynthesis of various class I heavy chains (*8*).

To investigate this mutant phenotype, we transfected HLA-A1 and -B8 complementary DNA (cDNA) constructs into 220 and control C1R cells, which also lack functional *HLA-A* and *-B* genes but are

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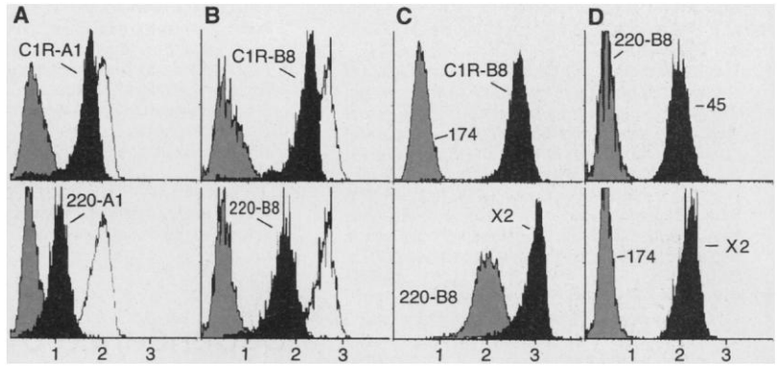

Fig. 1. Reduced surface expression of HLA-A1 and -B8 in 220-derived stable transfectants and mutual transcomplementation with mutant 174 cells after fusion (*22*). Surface amounts of HLA-A1 and -B8 were quantitated by indirect immunofluorescence and flow cytometry after binding of the specific mAbs GS142.1 and GSP8.1, respectively (*15*). (**A** and **B**) The average fluorescence intensities (logarithmic scale) of 220-A1 (A) and 220-B8 (B) cells were 20 and 25% of those seen with the C1R-A1 and C1R-B8 control transfectants, respectively. Independent transfectant isolates gave similar results. Shaded profiles show control stainings of untransfected C1R and 220 cells. Open profiles show binding of GS142.1 and GSP8.1 by untransfected wild-type LCL 721.112 control cells (*9*). (**C**) Transcomplementation in a 220-B8 × 174 hybrid cell line (X2) resulted in large surface amounts of HLA-B8. (**D**) Hybrid X2 cells showed completely restored surface expression of 174-derived HLA-B5 molecules, by binding mAb 4D12 in amounts similar to those bound by the parental wild-type LCL 721.45 control cells (*23*).

otherwise normal (*10*). All of the 220 stable transfectant isolates displayed only 20 to 25% of the surface amounts of HLA-A1 and -B8 measured on the C1R transfectants (Fig. 1, A and B) (*8*), although the class I heavy chains were synthesized at similar rates in all of the transfectants and other surface glycoproteins (such as HLA-DR, CD20, and the transferrin receptor) were present in amounts equal to those on normal B-LCL (*11*). Corresponding results were obtained after transfection of HLA-G cDNA into 220 and control LCL 721.221 (HLA-A^-, -B^-, and -C^-) cells (*9*, *11*). Thus, a specific defect in 220 cells similarly impaired the surface expression of diverse class I molecules encoded by the four functional MHC class I genes. In accord with previous data, this defect was independent of TAP, because mutual genetic transcomplementation in a 220-B8 × 174 hybrid cell line (X2) restored large surface amounts of HLA-B8 and of 174-derived HLA-B5 (Fig. 1, C and D) (*8*). Moreover, TAP-dependent translocation of a labeled reporter peptide into the ER of 220 cells was as effective as in the closely related control 221 cells and was more effective than in C1R cells (Fig. 2).

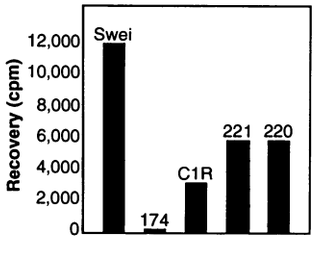

Fig. 2. Normal function of TAP in 220 cells. TAP-dependent peptide transport in 220 cells was as active as in control 221 cells and was more active than in C1R cells. Swei is an MHC-heterozygous B-LCL. Bars show recovery of a labeled reporter peptide with a glycosylation acceptor sequence. Peptides were introduced into cells by streptolysin O–mediated permeabilization of the outer cell membrane, and glycosylated peptides in the ER were isolated from cell extracts with concanavalin A–Sepharose beads as described (*24*). Data are representative of three experiments in which deviation was no more than ±10%.

To identify a functional defect, we examined the assembly of class I molecules in 220-B8 transfectant cells. The transient association of class I heavy chains with calnexin (IP90) was tested by immunoprecipitation of calnexin-glycoprotein complexes with the AF8 monoclonal antibody (mAb) from digitonin lysates of metabolically labeled cells (*12*). SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of these complexes gave similar band patterns for 220-B8 and C1R-B8 cells (Fig. 3A). The identity of putative class I heavy chain bands was confirmed by SDS dissociation of isolated calnexin complexes and secondary precipitation with mAb HC10, which binds free HLA-B and -C heavy chains (*13*). After pulse-labeling and chase, the intensities of the class I heavy chain bands decreased over the time of the chase at similar rates in the 220-B8 and C1R-B8 samples, which indicated their gradual dissociation from calnexin at the time of their association with β_2M (Fig. 3B).

We tested the peptide-dependent maturation of the class I heterodimers by quantitation of stably conformed HLA-B8 mol-

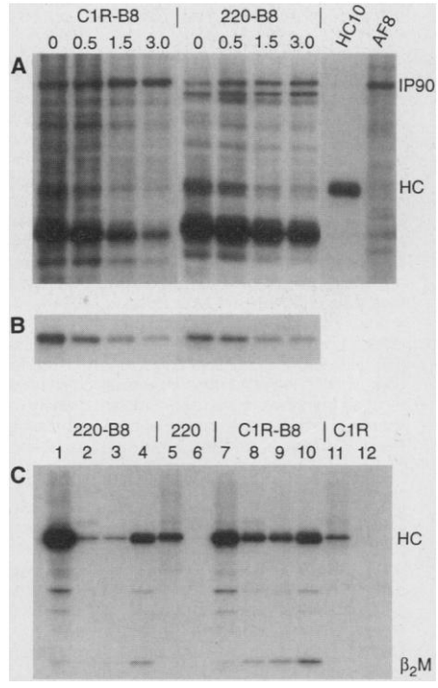

Fig. 3. Class I heavy chains associate normally with calnexin (IP90) in 220-B8 cells, but class I heavy chain–β_2M heterodimers fail to efficiently capture peptides. (**A**) Calnexin-glycoprotein complexes were isolated from 220-B8 and C1R-B8 control cells immediately after pulse labeling (0 hours), and at the indicated time points (0.5 to 3.0 hours) after the chase, by immunoprecipitation from digitonin lysates with mAb AF8 (*12*, *21*). The positions of HLA-B and -C heavy chains (HC) were identified by parallel direct precipitation with mAb HC10 from C1R-B8 lysate (lane HC10). Likewise, the single calnexin band (IP90) was identified in a parallel precipitation, after disruption of protein complexes from C1R-B8 lysate with SDS (lane AF8). (**B**) Bona fide HLA-B8 and -C heavy chains were recovered from SDS-dissociated AF8 immunocomplexes from reprecipitation with mAb HC10 (*21*). The intensities of the heavy chain bands decreased over the time of the chase at similar rates in the C1R-B8 and 220-B8 lanes. (**C**) Mutant 220-B8 cells contained unstable HLA-B8 heterodimers that were stabilized by specific peptide. Labeled HLA-B8 complexes were immunoprecipitated with mAb GSP8.1 from 220-B8 and C1R-B8 cell lysates after dissociation for 1 hour (lanes 2 and 8) and overnight (lanes 3 and 9) at 4°C (*14*, *25*). The heavy chain–β_2M heterodimers from 220-B8 cells dissociated rapidly but were stabilized when specific peptide was added to samples immediately after cell lysis (lanes 4 and 10) (*16*, *25*). GSP8.1 was specific for HLA-B8 as no bands were seen in the 220 and C1R control lanes (lanes 6 and 12) and was β_2M-dependent (lanes 2 through 4). Large amounts of labeled free HLA-B8 heavy chains were precipitated with mAb HC10 from 220-B8 and C1R-B8 control lysates (lanes 1 and 7). Control lanes 5 and 11 show the amounts of HLA-C heavy chains bound by HC10 in lysates of untransfected 220 and C1R cells.

ecules after dissociation of unstable complexes in lysates of metabolically labeled 220-B8 and C1R-B8 cells for 1 hour and overnight at 4°C (*14*). Samples were immunoprecipitated with mAb GSP8.1, which bound HLA-B8–β_2M complexes but not free heavy chains (*15*) (Fig. 3C). After SDS-PAGE, the HLA-B8 heavy chain and β_2M bands in the 220-B8 lanes were of much lower intensity than those in the C1R-B8 lanes (Fig. 3C, lanes 2 and 3 and 8 and 9), although similar amounts of radiolabeled heavy chains were present in all of the lysates (Fig. 3C, lanes 1 and 7). Thus, most of the HLA-B8 complexes from 220-B8 cells dissociated rapidly, whereas those from C1R-B8 cells were stable. However, they were effectively stabilized when a specific HLA-B8 binding peptide was added to lysate of 220-B8 cells (*16*) (Fig. 3C, lane 4). Hence, most of the HLA-B8 complexes from 220-B8 cells were devoid of peptides and were capable of peptide binding in vitro. This indicated that properly folded HLA-B8 heterodimers failed to bind appropriate peptides in 220-B8 cells.

These results suggested a defect preventing the access of class I heterodimers to peptides, possibly by abrogation of their ability to associate with TAP. Using a TAP1 antiserum, we immunoprecipitated TAP–class I heterodimer complexes from digitonin lysates of metabolically labeled cells (*4, 17*). As expected, SDS-PAGE revealed putative class I heavy chains with a molecular mass of 44 kD that were coprecipitated from C1R, C1R-B8, LCL 721.45, and LCL 721.112 cell lysates, but not from lysate of 174 cells (Fig. 4A). Bona fide HLA-B5 (45), -B8 (C1R-B8, 112), and -C (C1R) heavy chains were efficiently recovered from these complexes after SDS dissociation and reprecipitation with mAb HC10 (Fig. 4B). By contrast, HLA-B8 and -C heavy chains were completely missing in TAP complexes from 220-B8 and 220 cells (Fig. 4, A and B), respectively, although control lysates contained normal amounts of radiolabeled heavy chains and of TAP1 and TAP2 polypeptides (Fig. 4, C and D). Correspondingly, HLA-A1 and -G heavy chains were missing in TAP complexes from 220-A1 and 220-G cells, respectively, after secondary precipitation with mAb HCA2, which effectively bound these heavy chains in primary precipitations and in parallel secondary precipitations from dissociated TAP complexes isolated from the control C1R-A1 and 221-G cells (*13*) (Fig. 4, E and F). Thus, all of the four diverse class I heterodimers tested failed to associate with TAP in 220 cells. This deficiency correlated with the incomplete assembly and reduced surface expression of the class I molecules in this mutant, because TAP–class I heterodimer complexes were reconstituted in the X2 hybrid of 220-B8 and 174 cells (Fig. 4, A and B), in which large amounts of HLA-B8 at the cell surface were restored by transcomplementation (Fig. 1C). Thus, the ability of the class I heterodimers to bind peptides was highly dependent on their physical interaction with TAP, which was under distinct genetic control.

Similar to all human TAP-deficient mutant cell lines, mutant 220 cells originated from MHC-hemizygous parental cells with a deletion ranging from 6p11 to 6pter on the short arm of chromosome 6 that includes the MHC at 6p21 (*6–9, 18*). A probable association of the genetic defect in 220 cells with this hemizygous chromosomal region was indicated by the spontaneous reversion of a 220-B8 × 174 hybrid cell line to the mutant 220-B8 phenotype concomitant with loss of the single 174-derived MHC haplotype. With all of 60 individual revertants isolated by limiting dilution, small amounts of surface HLA-B8 correlated with the absence of surface HLA-A2 and -B5 encoded in the MHC of 174 cells (*9*). This observation was directly confirmed by fusion of 220-B8 cells with microcells prepared from the mouse-human monochromosomal hybrid cell line RA6A, which contains a human chromosome 6 tagged with the guanine phosphorybosyl-transferase (gpt)–dominant selectable marker (*19*). Isolates selected with mycophenolic acid for the stable maintenance of the transferred chromosome had large amounts of surface HLA-B8 (Fig. 5). Thus, a gene or genes controlling the TAP–class I heterodimer interaction were linked to the MHC on chromosome 6, with a highly probable location between 6p11 and 6pter on the short arm of this chromosome.

These results demonstrate that class I heterodimers must associate with TAP to gain access to their natural cytosol-derived peptide ligands. This requirement may not be absolute, because thermostable class I molecules are present on the surfaces of 220 transfectant cells cultured at 37°C. However, it is unknown whether the peptides bound by these molecules are of cytosolic origin, and a small proportion of class I heterodimers may associate with TAP in 220 cells. Because class I molecules are capable of binding peptides experimentally introduced into the ER in a TAP-independent manner by fusion to a hydrophobic signal sequence (*20*), the failure of most class I heterodimers to bind peptides in 220 cells indicates that peptides supplied by TAP are scarce in the lumen of the ER of normal cells. In previous transfections of 220 cells, the surface amounts of some class

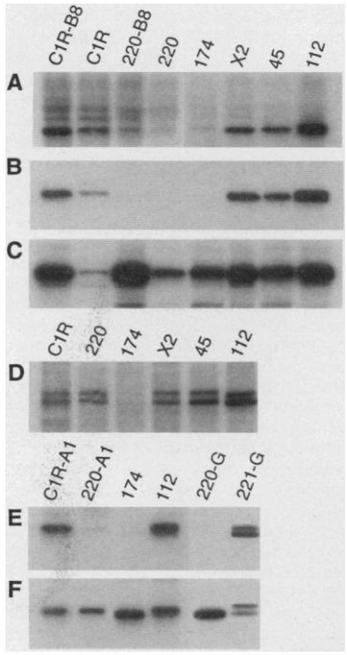

Fig. 4. Class I heterodimers fail to associate with TAP in mutant 220 cells. (**A**) Putative class I heavy chains were coprecipitated with TAP proteins by a TAP1 rabbit antiserum from digitonin lysates of several cell lines (*17, 26*). Only faint bands were seen in the lanes of 220-B8, 220, and 174 (TAP$^-$) cells. (**B**) After dissociation of the TAP–class I complexes and secondary precipitation with HC10, the 220-B8, 220, and 174 lanes were almost equally blank, although HLA-B and -C heavy chains were efficiently recovered in the other lanes (*26*). (**C**) Primary precipitations with HC10 showed the relative amounts of labeled HLA-B or -C heavy chains present in all of the cell lysates. (**D**) Bands corresponding to the TAP1 and TAP2 subunit proteins (lower and upper bands, respectively) (*6*) were missing only in lysate of 174 cells. (**E**) HLA-A1 and -G heavy chains were missing in TAP complexes isolated from 220-A1 and 220-G cell lysates after secondary precipitation with mAb HCA2, although they were efficiently recovered from TAP complexes isolated from the C1R-A1 and 221-G control cells. (**F**) In a parallel experiment, free heavy chains were bound by HCA2 in all cell lysates.

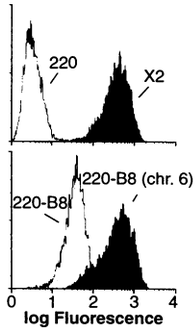

Fig. 5. A gene or genes controlling the TAP–class I heterodimer interaction are linked to the MHC on chromosome (chr.) 6. Stable transfer by microcell fusion of a human gpt-tagged chromosome 6 from the mouse-human hybrid cell line RA6A into 220-B8 cells restored surface levels of HLA-B8 (lower filled profile) that were as high as on the X2 hybrid cell line (upper filled profile) (*19, 27*). Open profiles are control stainings of 220 and 220-B8 cells. Cells were examined by indirect immunofluorescence with mAb GSP8.1 and flow cytometry (*15*).

I molecules inconsistently varied within wide ranges. These variances were almost certainly the result of expression of the class I heavy chains far above physiological levels because of the use of nonintegrating episomal vectors and the variation in the copy number of these constructs among different transfectants (8). Our results indicate that the TAP–class I interaction provides an adaptive mechanism that promotes the efficient capture of cytosolic peptides by presumably all diverse class I heterodimers. This complex interaction is controlled by an unidentified gene or genes linked to the MHC, which encodes an accessory molecule (or molecules) that may regulate the delivery of class I heterodimers to TAP or their association with TAP.

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21. C1R-B8 and 220-B8 cells (1.8×10^7 and 1.2×10^7, respectively) were metabolically labeled with [^{35}S]methionine [0.5 mCi/10^7 cells/1 ml of methionine-free RPMI 1640 media (ICN) supplemented with 10% dialyzed fetal calf serum (FCS) (Hyclone)] for 5 min. For the chase, a 10-fold excess of complete media [RPMI 1640 and 10% FCS (Gibco)] was added. Aliquots of 3×10^6 cells were withdrawn before the chase and at the indicated time points during the chase. Cells were washed with cold phosphate-buffered saline and lysed in 0.5 ml of cold digitonin lysis buffer (DLB) [0.5% digitonin (Aldrich), recrystallized, 25 mM $CaCl_2$, 5 mM iodoacetamide, and 2 mM phenylmethylsulfonyl fluoride]. Lysates were preadsorbed with formalin-fixed *Staphylococcus aureus* bacteria (Sigma), and calnexin complexes were precipitated with 2 μl of AF8 ascites. Immunocomplexes were collected with protein A–Sepharose CL-4B (Pharmacia) and washed repeatedly with cold DLB. In parallel, the HC10 and AF8 control precipitations were carried out each with 3×10^6 C1R-B8 cells immediately after labeling. HC10 purified from ascites was used at a concentration of 7 μg per 0.5 ml of lysate. For the AF8 control precipitation, labeled C1R-B8 cells were lysed in 50 μl of DLB and 0.2% SDS. After 30 min at 4°C, 0.45 ml of DLB was added and the sample was processed as above. For the secondary precipitations, half of the primary AF8 immunocomplexes bound to protein A–Sepharose from each time point sample were incubated in 50 μl of TNE [50 mM tris-OH (pH 7.5), 150 mM NaCl, and 5 mM EDTA] and 0.2% SDS for 1 hour at 37°C. Samples were then adjusted to 0.5 ml of TNE containing 1% NP-40 (Pierce) and 0.5% Mega-9 (Sigma). Protein A–Sepharose immunocomplexes were removed by centrifugation, and class I heavy chains were reprecipitated from the supernatants with HC10. After binding to protein A–Sepharose, immunocomplexes were washed with TNE and 0.5% NP-40. Denatured samples were analyzed by SDS-PAGE (10% acrylamide). Fixed gels were treated with Amplify (Amersham) and dried for autoradiography.
22. Transfections of 220 and C1R cells with HLA-A1 and -B8 cDNAs in RSV.5neo [M. DiBrino *et al.*, *J. Immunol.* **152**, 620 (1994)] were carried out with a Gene Pulser (Bio-Rad), using settings of 210 V and 960 μF for 220 cells, and 250 V and 500 μF for C1R cells (*7*). Transfectants were selected in 24-well plates, with G418 (Gibco) (600 μg/ml with 220 cells and 1.8 mg/ml with C1R cells). HLA-G cDNA was stably introduced into 220 and 221 cells with the use of a retroviral construct [T. Fujii, A. Ishitani, D. E. Geraghty, *J. Immunol.* **153**, 5516 (1994)]. X2 hybrid cells were isolated after standard polyethylene glycol–mediated fusion of 220-B8 and 174 cells stably transfected with pREP8Δ (*7*). Hybrids were plated on irradiated MRC-5 fibroblasts and selected with G418 (600 μg/ml) and L-histidinol (2.5 mM) (Sigma) (*7*). For flow cytometry, cells were stained with the specific mAbs purified from ascites and with phycoerythrin-conjugated goat antibody to mouse $F(ab')_2$ (Tago) and examined with a Becton Dickinson FACScan flow cytometer.
23. B. F. Haynes, E. G. Reisner, M. E. Hemler, J. L. Strominger, G. S. Eisenbarth, *Hum. Immunol.* **4**, 273 (1982).
24. M. J. Androlewicz and P. Cresswell, *Immunity* **1**, 7 (1994).
25. Cells were metabolically labeled for 45 min with the use of 0.2 mCi of [^{35}S]methionine/10^7 cells/1 ml of methionine-free RPMI labeling media (*21*). About 5×10^6 labeled cells were used for each sample lane. Incorporated radioactivity was determined for all samples by acid precipitation of crude lysate aliquots. Lysates were prepared and processed exactly as described (*14*). Peptide FLRGRAYGI (*16*) (purified by high-performance liquid chromatography) was used at a concentration of 50 mM. GSP8.1 and HC10 mAbs were purified from ascites and used at a final concentration of 15 μg/ml of lysate. Samples in proportion to total incorporated radioactivity were subjected to SDS-PAGE (12% acrylamide).
26. In Fig. 4, A through C and E and F, cells were labeled for 20 min as described (*21*). Aliquots of 3×10^6 and 6×10^6 labeled cells were used for Fig. 4, A, C, D, and F, and Fig. 4, B and E, respectively. The conditions and primary and secondary immunoprecipitation procedures were as described (*21*). Three milliliters of the TAP antiserum was used for each sample. Purified HC10 and HCA2 were used at a final concentration of 15 μg per 1 ml of cell lysate. In Fig. 4D, cells were labeled for 45 min and lysed in TNE with 0.5% NP-40 and protease inhibitors (*21*). After removal of nuclei, lysates were adjusted to a concentration of 1% sodium deoxycholate and 0.1% SDS. TNE with these detergents was also used for washing of the immunocomplexes bound to protein A–Sepharose in Fig. 4D. Before analysis by SDS-PAGE (10% acrylamide), samples from each experiment were adjusted by volume in proportion to the radioactivity that was acid-precipitated from the crude lysates.
27. Semiconfluent mouse-human RA6A hybrid cells were exposed to colcemide for 36 hours to induce the formation of micronuclei. Cells were centrifuged in Percoll–cytochalasin B to shear microcells and collected [E. Stubblefield and M. Pershouse, *Somatic Cell Mol. Genet.* **18**, 485 (1992)]. Microcells and 220-B8 cells were fused at a ratio of 10:1 in 50% polyethylene glycol, and cell hybrids containing the gpt-tagged chromosome 6 were selected in 24-well plates with mycophenolic acid (25 μg/ml) and xanthine (70 μg/ml) (*19*). RA6A cells were selected with G418 (600 μg/ml).

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