CD4 was present in cosedimenting membrane vesicles derived from quail cells, we performed control experiments in which CD4 was expressed in uninfected quail QT6 cells. We observed concentrations of CD4 in the media approximately 5% of those found in the media from ALV-infected cells; since CD4 from the media of uninfected cells migrated at a density of 1.12 to 1.14 g/ml in linear sucrose gradients (*4*), we conclude that CD4 in these control experiments is contained in less dense membrane-derived vesicles.

The incorporation of CD4 into ALV particles was an unexpected result, because cell surface proteins are generally not efficiently assembled into virus particles. Indeed, most quail cell glycoproteins were excluded from ALV particles in our experiments (Fig. 2). Since human CD4 is normally expressed in lymphocytes, it seems likely that quail (QT6) fibroblast cells lack the proteins that normally interact with CD4 (for example, $p56^{lck}$) (*8*). Consequently, CD4 might differ from other glycoproteins at the surface of quail cells because it lacks interactions with other proteins. This raises the possibility that virus incorporation of host cell surface proteins might be prevented, at least in part, by the interactions between these proteins and cytoplasmic proteins.

At present we cannot explain why CR and CM proteins were assembled into ALV particles much less efficiently than was wild-type human CD4. However, we have obtained an intriguing result by transiently expressing CD4 and CR proteins in quail [R(-)Q No. 3] cells that contain an avian sarcoma virus genome lacking *env* (*9*). Virus particles produced from these cells incorporate CD4 or CR proteins with equal efficiencies (*4*). This suggests that Env proteins might compete with chimeric proteins, but not with CD4 proteins, for assembly into ALV particles. Alternatively, interactions between chimeric proteins and viral Env proteins might prevent their incorporation into virus particles.

CD4 is the first example of a nonviral cell surface protein with transmembrane and cytoplasmic tail domains shown to be efficiently incorporated into retroviral particles. Other studies have shown that Thy-1, which is attached to the cell surface by a glycosylphosphatidylinositol linkage (*10*), is incorporated into MLV particles (*11*). The uptake of cell surface proteins by virus particles could influence the host cell tropism of a virus if interactions between these proteins and their cognate receptors can direct virus infection of normally resistant cell types. This suggests a strategy to target viral vectors containing therapeutic genes to specific cell types. For example, since CD4 and the gp120 Env proteins of human immunodeficiency viruses type–1 and type–2 (HIV-1 and HIV-2) interact with high affinity (*12*), ALV (CD4) pseudotypes can be used to test if virus-associated CD4 proteins can target ALV vectors to cells expressing HIV Env glycoproteins. If so, it might be possible to use this sytem to specifically deliver retroviral vectors containing dominant negative mutations in *gag*, *tat*, or *rev* genes (*13*) to cells infected with HIV.

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17. The chicken β-actin promoter (provided by S. Hughes) was introduced into the Eco RI site of plasmid pSV7d (*16*) to generate a plasmid designated pSVβ (P. Bates, unpublished data).
18. QAH cells are quail (QT6) cells infected with a replication-competent ALV vector, RCASH, derived from the RCAS vector [S. Hughes *et al.*, *J. Virol.* **61**, 3004 (1987)] containing, at the Cla I cloning site, a hygromycin B resistance gene driven by the thymidine kinase promoter of herpes simplex virus (P. Bates, J. A. T. Young, H. E. Varmus, unpublished data).
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The Role of β_2-Microglobulin in Peptide Binding by Class I Molecules

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Efficient transport of class I major histocompatibility complex molecules to the cell surface requires association of the class I heavy chain with endogenous peptide and the class I light chain, β_2-microglobulin (β_2M). A mutant cell line deficient in β_2M transports low amounts of nonpeptide-associated heavy chains to the cell surface that can associate with exogenously provided β_2M and synthetic peptide antigens. Normal β_2M-sufficient cells grown in serum-free media devoid of β_2M also require an exogenous source of β_2M to efficiently bind synthetic peptide. Thus, class I molecules on normal cells do not spontaneously bind or exchange peptides.

CYTOLYTIC T LYMPHOCYTES (CTLs) recognize peptide antigens bound within the antigen-binding groove of class I molecules (*1–4*). Peptides derived from processing of endogenously synthesized proteins become associated with class I molecules during their assembly in the endoplasmic reticulum (*5, 6*). Studies of mutant cell lines indicate that efficient transport of class I to the cell surface requires association of the class I heavy chain with both peptide and β_2M (*6–8*). Once expressed on the cell surface, class I molecules can bind or exchange peptide, as demonstrated by the ability of glutaraldehyde-fixed cells (*9*) or purified class I molecules (*10, 11*) to bind and present exogenously provided synthetic peptides for recognition by CTLs. However, this exchange is inefficient, as measured by the small percentage of purified class I molecules that can bind peptide (0.3%)

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Table 1. Expression of class I molecules by 439β_2M^{del} cells. Flow cytofluorometry analysis of class I expression by cells grown in media containing 10% fetal bovine serum or 1% serum-free (SF) media (Nutridoma, Boehringer Mannheim). Cells (5×10^5) were incubated for 90 min on ice with MAb (50 μl) in the form of hybridoma supernatant, washed twice in Hank's balanced salt solution, and incubated with fluorescein isothiocyanate–labeled rabbit $(Fab')_2$ to mouse immunoglobulin (2 μg; Zymed) for 45 min on ice. Samples were washed twice and analyzed on a Becton-Dickinson FACS 440. Numbers represent the mean linear fluorescence of 10,000 events. Results comparable to those reported above have been obtained in two or more independent experiments.

Antibody	Cell line (fluorescence units)				
	R1E/D^b	439β_2M^{del}	439.4.2	439β_2M^{del} − SF	439-SF
(−)	5.4	4.0	5.5	3.2	5.1
28-11-5S (D^b)	5.5	8.3	1180	4.5	732
28-14-8S (D^b,L^d)	102	15.0	781	6.2	450
Y3 (K^b,K^k)	5.4	4.9	466	3.1	229
34-2-12S (D^d)	5.5	4.7	379		
12-2-12S (K^k)	5.6	4.4	5.1		
34-1-2S (K^d,D^d)	5.7	5.4	1116		

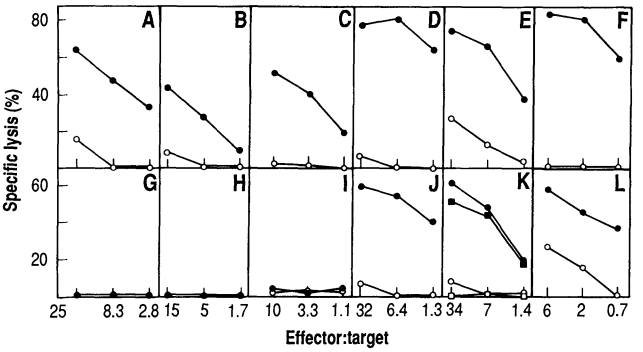

Fig. 1. Lysis of normal and β_2M-deficient cells. 439.4.2 cells (**A** to **F**), the β_2M-deficient cell line 439β_2M^{del} (**G** to **J**, and **L**), or R1E/D^b (**K**) were either infected with influenza virus A/PR/8/34 (A, B, G, and H), loaded with OVA by pinolysis (C and I), coated with peptide NP(147-158R^-) (*3*) (D and J), coated with peptide NP(365-380) (E and K), or coated with peptide OVA (253-276) (F and L) and used as targets for lysis by antigen-specific CTLs. (●), Infected or peptide-pulsed 439.4.2 (A to F) or 439β_2M^{del} (G to L); (○), untreated 439.4.2 (A to F) or 439β_2M^{del} (G to L); (■), peptide-pulsed R1E/D^b; (□), untreated R1E/D^b. Target cells were grown in RPMI 1640 supplemented with fetal bovine serum (10%), 2 μM glutamine, 5×10^{-5} M β-mercaptoethanol, and gentamicin (50 μg/ml) (culture media). For viral infection, cells were washed twice in RPMI 1640, and 30 hemagglutination units (HAU) of infectious virus in the form of allantoic fluid and ^{51}Cr (200 μCi) were added to 1×10^6 to 2×10^6 cells. Target cells were loaded with OVA protein by pinolysis as described (*4*), without the 6-hour incubation before addition of ^{51}Cr. Peptide-coated targets were prepared by adding ^{51}Cr to 1×10^6 to 2×10^6 cells in culture media (100 μl) with peptide (10 μg). After incubation for 90 min at 37°C, cells were washed four times and resuspended at 3×10^5/ml in culture media. In addition, OVA(253-276)–coated cells were exposed to peptide by previous overnight incubation with peptide (100 μM) in culture media. Effectors were a CTL population obtained by in vitro stimulation of spleen cells from BALB/c (A and G) or C57BL/6 (B and H) mice previously primed with virus, or an NP(147-158 R^-)–specific K^d-restricted CTL line clone 9 (D and J), an NP(365-380)–specific D^b-restricted line clone 34 (E and K), or the OVA-specific K^b-restricted line B3 (C, F, I, and L). Derivation of CTL populations and clones and assay conditions were as described (*17, 20*). For each experimental condition, similar results were obtained in at least three independent experiments.

(*10*). Here, we have used both β_2M-deficient and normal cells to examine the role of β_2M in peptide binding by class I molecules.

A β_2M-deficient cell line, 439β_2M^{del}, which expresses both H-2^d and H-2^b class I molecules, was derived from the (BALB/c × C57BL/6)F_1 Abelson-transformed cell line 439.4.2, with a two-step selection protocol. A β_2M^b-specific monoclonal antibody (MAb) and complement were used to remove cells expressing the β_2M^b allele, which resulted in numerous mutants with the 5′ end of the gene deleted (*12*). One of these mutants was then treated with a mixture of H-2^d– and H-2^b–specific MAbs and complement to remove cells with class I expression. A clone that survived this selection, 439β_2M^{del}, expressed no β_2M and had a deletion at the β_2M^a allele (*13*). As anticipated for a β_2M-deficient line, these cells expressed little class I (*8, 14, 15*) and were detected best with 28-14-8S, a MAb that binds the free heavy chains of D^b and L^d (*6, 14–16*) (Table 1). This result is consistent with previous observations that D^b heavy chains were expressed on the surface of a β_2M-deficient cell line, R1E, that was transfected with the gene encoding D^b (*14*). However, 439β_2M^{del} cells expressed much less D^b than the R1E/D^b transfectant. None of the other class I–specific MAbs used bind free class I heavy chains.

To determine if the low numbers of class I molecules on 439β_2M^{del} cells were sufficient to present antigen for CTL recognition, we infected 439β_2M^{del} cells with influenza virus and then tested for susceptibility to lysis by populations of influenza-specific CTLs. Although 439β_2M^{del} cells were successfully infected [surface expression of viral proteins (*13*)], they were not recognized by virus-specific CTLs restricted by H-2^d or H-2^b class I molecules (Fig. 1, G and H). Nor were they recognized by an ovalbumin (OVA)-specific K^b-restricted CTL clone (B3) after cells were loaded with this protein by osmotic lysis of pinocytic vesicles (*4, 17*) (Fig. 1, I). In contrast, the β_2M-positive parental line 439.4.2 effectively presented each of these endogenous antigens for recognition by CTLs (Fig. 1, A to C). The 439β_2M^{del} cells pulsed with synthetic peptides that contained the epitopes recognized by influenza nucleoprotein (NP)–specific CTL clones (*2, 3*) were able to present both K^d- and D^b-restricted (NP) peptides for recognition by NP-specific CTLs (Fig. 1, J and K). These same CTL clones did not recognize influenza virus–infected 439β_2M^{del} cells (*13*). The 439β_2M^{del} cells pulsed with a K^b-restricted OVA peptide could also be recognized by clone B3 (Fig. 1L); however, optimal lysis required overnight incubation of 439β_2M^{del} cells with the OVA peptide. Thus, the low amount of class I expressed on the surface of 439β_2M^{del} cells was sufficient for presentation of exogenous peptides to CTLs. As anticipated from these results, the R1E/D^b cell line was able to present the D^b-restricted NP peptide for recognition by the appropriate CTL clone (Fig. 1K).

These results suggested either that free class I heavy chains expressed on 439β_2M^{del} and R1E/D^b cells could bind and present exogenously provided peptide, or, alternatively, that β_2M present in the fetal bovine serum used as a media supplement was able to participate in formation of a class I–peptide complex. To distinguish between these possibilities, we adapted 439β_2M^{del} cells to growth in serum-free media and then assayed for peptide presentation. When 439β_2M^{del} cells were grown and pulsed with peptide in media deficient in β_2M, no lysis was observed (Table 2, experiment A). Lysis could be obtained if cells were pulsed with peptide in the presence of purified human β_2M. Further evidence that β_2M was required for peptide binding was obtained by testing the ability of β_2M-deficient serum to promote peptide binding. Removal of 80 to 90% of β_2M from human serum by affinity chromatography with a MAb to human β_2M, BBM.1, substantially dimin-

ished its ability to promote peptide binding by 439β_2M^{del} cells (Table 2, experiment B).

This requirement for β_2M could reflect the inability of heavy chain to bind peptide in the absence of β_2M, the inability of CTL to recognize a heavy chain–peptide complex that did not contain β_2M, or both. Because the cytolytic assays were performed in media supplemented with fetal bovine serum (and thus β_2M), the requirement for β_2M probably was at the time of peptide pulsing. The 439β_2M^{del} cells were incubated overnight in serum-free media containing 56 μM peptide, and then washed to remove free peptide before incubation in media containing β_2M (Table 2, experiment C). Despite prolonged exposure to a high concentration of peptide, no lysis was observed. In contrast, cells incubated overnight in media containing β_2M and washed free of β_2M before pulsing with peptide were specifically lysed by CTLs. The presence of β_2M was detectable by BBM.1 (mean fluorescence 10.4 over a background of 5.1). Thus, β_2M but not peptide could stably associate with the class I heavy chain on the cell surface. Only during or after association with β_2M could heavy chain stably bind peptide. This inability of class I heavy chains to stably bind peptide is consistent with the inability of 439β_2M^{del} cells to present endogenous antigens for recognition by CTLs (Fig. 2).

To estimate the number of class I molecules required for T cell recognition and lysis, we performed fluorescence-activated cell sorter (FACS) analysis on cells grown under conditions used for the cytolytic assay (Fig. 1). By comparing the amount of fluorescence on 439β_2M^{del} cells grown in serum with that observed for 439.4.2, we estimated that K^d, K^b, and D^d were each expressed on 439β_2M^{del} at less than 0.2% of the amount found in 439.4.2 (Table 1), in which class I has been estimated to be approximately 100,000 molecules per cell (*18*). The D^b-specific monoclonal 28-11-5S, which binds those D^b molecules associated with β_2M, detected fewer than 0.4% of the number of molecules expressed on 439.4.2. These results suggest an upper limit for the number of class I molecules required for CTL recognition in the range of 200 to 400. The number of class I molecules on 439β_2M^{del} cells could not be increased by incubating cells in the presence of peptides that bind K^d, D^b, or K^b (*13*).

As a control, we assessed the ability of the nonmutant β_2M^+ line 439.4.2 to present exogenous peptide when grown and pulsed with peptide in serum-free media. Surprisingly, peptides were presented much less efficiently by cells pulsed in serum-free media than by cells pulsed in media supplemented with fetal bovine serum (Fig. 2, A and C). Addition of purified β_2M at the time of pulsing with peptide markedly enhanced the efficiency of peptide association with either K^d or D^b (Fig. 2, B and D). Thus, class I molecules on normal cells do not spontaneously bind or exchange exogenously provided peptides, perhaps because they are already occupied with endogenous peptides. The β_2M may promote the binding of peptide to normal cells, as it does to β_2M-deficient cells, by its association with free class I heavy chains, thereby providing "empty" class I molecules capable of binding exogenous peptide. Alternatively, because free β_2M readily exchanges with β_2M bound by class I molecules (*19*), it is also possible that β_2M exchange facilitates peptide exchange. Serum was more efficient than purified β_2M at promoting peptide binding by class I molecules on normal cells (Fig. 2); thus, another component in serum may further promote peptide binding or exchange by class I molecules.

Results similar to those obtained with 439.4.2 have been obtained with other cell lines that have been tested, including the murine tumor lines P815 and EL4 (*13*). Cells must be grown in serum-free media for 1 to 2 weeks before a requirement for β_2M is observed. One cell line tested, $T2K^b$ (*7*), was able to bind peptide in the absence of exogenous β_2M or serum. Because $T2K^b$ is a mutant line that is believed to transport empty β_2M-associated class I molecules to the cell surface, this finding supports the hypothesis that class I molecules on normal cells do not spontaneously bind exogenous peptides because they are already occupied.

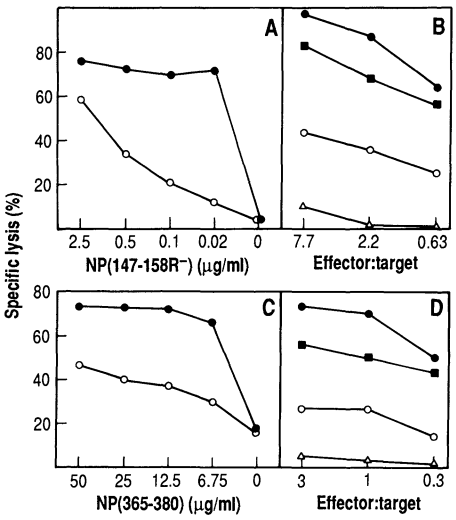

Fig. 2. Exogenous β_2M enhances the efficiency of peptide–class I association on 439.4.2 cells in serum-free media. (**A** and **C**) 439.4.2 cells were grown and pulsed with the indicated concentration of peptide in media supplemented with either fetal bovine serum (●) or serum substitute (○) and used as targets with CTL clone 9 at an effector to target ratio of 2:1 (A) or CTL clone 34 at an effector to target ratio of 2.5:1 (C). (**B** and **D**) 439.4.2 cells were grown in media with serum substitute and pulsed with peptide in media containing serum substitute (○), supplemented with human β_2M (3 μg/ml) (■), or supplemented with fetal bovine serum (10%) (●); (△), no peptide. Cells were pulsed with NP(147-158 R^-) (2 μg/ml) and used as targets for CTL clone 9 (B) or pulsed with NP(365-380) (10 μg/ml) and used as targets with CTL clone 34 (D). For each experimental condition, similar results were observed in at least three independent experiments.

Table 2. Lysis of 439β_2M^{del} cells grown in serum-free media requires β_2-M. 439β_2M^{del} cells were cultured overnight in media containing serum substitute, washed twice, and then incubated for 2 hours with ^{51}Cr. Where indicated, NP(365-380) (56 μM), human β_2M (3 μg/ml; Sigma), or 20 μl of human serum-depleted β_2M was added during overnight incubation or ^{51}Cr labeling. CTL clone 34 was used at the indicated effector to target ratio to detect formation of the D^b-peptide complex. Cytolytic assays were performed as described in Fig. 1. Results similar to these were also obtained in two other independent experiments.

Experiment	Addition overnight		Addition during 2-hour pulse			Specific release (%)	3:1
	NP(365-380)	β_2M	NP(365-380)	β_2M	Serum − β_2M	10:1	
A	−	−	−	−	−	23	14
	−	−	+	−	−	21	12
	−	−	−	+	−	21	15
	−	−	+	+	−	55	25
B	−	−	−	+	+	21	13
	−	−	+	−	+	34	27
	−	−	+	+	+	68	59
C	−	−	−	−	−	23	14
	+	−	−	−	−	25	22
	−	+	−	−	−	24	20
	+	+	−	−	−	48	39
	+	−	−	+	−	21	21
	−	+	+	−	−	61	53

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Disruption of the Human SCL Locus by "Illegitimate" V-(D)-J Recombinase Activity

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A fusion complementary DNA in the T cell line HSB-2 elucidates a provocative mechanism for the disruption of the putative hematopoietic transcription factor *SCL*. The fusion cDNA results from an interstitial deletion between a previously unknown locus, *SIL* (*SCL* interrupting locus), and the 5′ untranslated region of *SCL*. Similar to 1;14 translocations, this deletion disrupts the *SCL* 5′ regulatory region. This event is probably mediated by V-(D)-J recombinase activity, although neither locus is an immunoglobulin or a T cell receptor. Two other T cell lines, CEM and RPMI 8402, have essentially identical deletions. Thus, in lymphocytes, growth-affecting genes other than immune receptors risk rearrangements.

THE *SCL* (ALSO CALLED *TCL5* AND *tal*-1) gene is a member of the family of genes defined by a primary amino acid motif consisting of a basic domain (B) NH_2-terminal to a helix-loop-helix (HLH) structure (*1, 2*). This family of proteins participates in either the growth or differentiation of the tissues in which its members are expressed. In many cases the B-HLH motif confers both cell type–specific DNA binding as well as protein-protein dimerization capability on the encoded gene product (*3*). *SCL* was originally identified at the site of a 1;14 translocation associated with the development of a hematopoietic "stem" cell leukemia in a 16-year-old male (*4*). The *SCL* transcript is in developing human fetal liver and adult regenerative bone marrow, but it is not in more mature cells of the lymphoid or myeloid lineages. Because of its (i) association with a stem cell leukemia, (ii) pattern of expression, and (iii) identification as an HLH family member, it probably is a hematopoietic transcription factor involved in early hematopoiesis. Other 1;14 translocations involving the *SCL* locus have been characterized (*2, 5*) and are associated with the development of both stem cell and less mature T cell leukemias. All of the translocation breakpoints either structurally or functionally eliminate a portion of the 5′ untranslated region of the *SCL* message (*6*).

While studying the *SCL* transcript, we used the anchored polymerase chain reaction (PCR) technique (*7*) to clone cDNA from the T cell line HSB-2 (*8*). Sequence analysis of a subset of the HSB-2 cDNA clones revealed a novel 5′ exon. One of these clones was used as a probe in a ribonuclease (RNase) protection assay and was completely protected in the HSB-2 cell line (Fig. 1A). In some other tissues and cell lines, including those that do not express *SCL*, only the novel exon was protected, suggesting that the HSB-2 cDNAs were unlikely to represent a normal, common *SCL* variant. We used the same probe to screen a human bone marrow cDNA library. Two nonoverlapping sets of clones were obtained. One set belonged to *SCL*, and the other set contained the novel 5′ exon of the HSB-2 cDNA, no *SCL* homology, and was otherwise unique. The cDNA identified by the novel 5′ exon hybridized to a 5.5-kb mRNA from thymus and the T cell line SUPT1 (Fig. 1B), neither of which express the normal 5-kb *SCL* message. Thus, in the cell line HSB-2, two normally distinct transcripts, one of which was derived from *SCL*, had apparently become fused. The bulk of *SCL*, including the complete *SCL* coding sequence, had been retained in the fusion message, but the *SCL* 5′ end had been replaced by an exon that was normally found in a distinct locus (Fig. 2). We called this newly identified region "SIL" for SCL interrupting locus. As with *SCL*, *SIL* is conserved cross-species (*9*). We have not found any significant identity between *SIL* sequences and any gene sequence previously submitted to GenBank. It is unlikely that a chimeric *SIL/SCL* protein is formed, because *SIL* joins *SCL* in the *SCL* 5′ untranslated region upstream of an in-frame TAA stop codon that precedes the initiation ATG in the *SCL* message.

To analyze the formation of this fusion gene, we digested HSB-2 genomic DNA with Bam HI, Eco RI, or Hind III and probed with probe 2.2XX (Fig. 3A). Rearranged bands were seen in all three digests (the Hind III digest is shown in the left panel of Fig. 3B). These rearrangements are not seen in the SB cell line (*10*), a B lymphoblastoid line derived from the same patient as HSB-2 (*11*). Thus, the rearrangements are neither polymorphisms nor constitutional rearrangements. Using an *SIL*

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