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Analysis of the Stability of HLA-A2 Molecules Expressed on the Cell Surface

Jong-Seok Lim†, Ki-Young Lee†, Hee Gu Lee, Ik-Hwan Kim, Chong Kil Lee[1], Seong Sun Han[1] and Kilhyoun Kim[2]*

Korea Research Institute of Bioscience and Biotechnology, Taejon 305-600, [1]Chungbuk National University, Cheongju 360-763 and [2]Department of Pharmacy, Ewha Womans University, Seoul 120-750, Korea
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Abstract: Association of antigenic peptide with class I MHC is believed to be crucial for maintaining stable conformation of class I molecules. T2 cells that are defective in TAP gene function mainly express class I molecules with an unstable conformation due to little or no association with antigenic peptides, whereas T1 cells that are normal in TAP gene function mainly express the stable form of class I molecules. In this work, attempts were made to determine the molecular stability of stable and unstable class I molecules. Dissociation of HLA-A2 molecules on T1 and T2 cells was monitored by flow cytometry using anti-HLA-A2 antibody after the cells were treated with brefeldin A to shut down the transport of newly-assembled HLA-A2. Estimated dissociation rate constants for the stable and unstable forms of HLA-A2 were 0.076 h^{-1} and 0.66 h^{-1}, respectively. It appeared that both T1 and T2 cells express stable and unstable class I complex, but with different ratios of the two forms. Furthermore, interferon-γ treatment of T1 cells appeared to induce the expression of both the stable and unstable class I molecules. These results demonstrate that class I MHC molecules can be divided into two groups in terms of structural stability and that they exist on the cell surface in both forms in a certain ratio.
Key words: cell surface expression, dissociation rate constant, HLA-A2 molecule, interferon-γ.

HLA class I molecule consists of an MHC-encoded α chain and a β2-microglobulin molecule (β2m). There is usually an antigenic peptide of 8~10 amino acid residues in the peptide binding groove (Townsend *et al.*, 1989; Townsend *et al.*, 1990; Neefies *et al.*, 1992). The α, or heavy chain, which includes peptide binding domains α1 and α2, can exist on the cell surface in three different forms: free α chain, a binary complex with a β2m, or a ternary complex associated with a β2m and a peptide (Williams *et al.*, 1989; Rock *et al.*, 1991). The "empty" binary complexes are often found on peptide transporter-deficient cell lines like T2. The T2 cells were produced by fusing a B lymphoblastoid cell line (.174) with a homozygous deletion of the class II region of the MHC to T lymphoblastoid cells (CEM) (De Mars *et al.*, 1984; Salter *et al.*, 1985; Salter *et al.*, 1986). The peptide transporters, designated TAP-1 and TAP-2, are deleted in the T2 cells and thus the cells are unable to properly assemble the MHC class I molecules, resulting in a low level or absent expression of HLA-A2 and -B5; another hybridoma T1 obtained from the fusion is normal in terms of TAP gene functions and thus expresses normal level of HLA molecules (Spies *et al.*, 1991; Kelly *et al.*, 1992). T2 cells also fail to process and present antigen after virus infection. It has also been reported that initial assembly of the heavy chain and β2m apparently takes place normally in T2, but yields conformationally-labile complexes which can be stabilized by the exogenous addition of appropriate peptides (Hoskin and Bevan, 1990; Anderson *et al.*, 1991; Baas *et al.*, 1992). The earlier studies interestingly showed that less than 1% of the HLA-A2 molecules on the intact cell surface could form complexes with high-affinity peptides, which was then much more stable (Chen and Parham, 1989; Tsomides *et al.*, 1991). These results suggest that there is always a certain amount of receptive class I molecules on the cell surface and the size of this population may depend on the MHC allele and cell type used.

Although it has been clearly shown that T2 cells express the unstable complexes of class I, they also express a significant amount of stable complexes of class I molecules (Cerundolo *et al.*, 1990). What fractions of the surface class I molecules are in stable or in un-

†These authors contibuted equally to the work contained in this manuscript.

*To whom correspondence should be addressed.
Tel/Fax: 82-2-360-2549.

stable conformation is largely unknown. Moreover, it has not been quantitatively described how much more stable the former is than the latter. In this work, we determined the kinetic characteristics of the stable and unstable class I molecules expressed on the surface of T1 and T2 cells after treatment with brefeldin A (BFA) to shut down the transport of newly-assembled class I from endoplasmic reticulum (ER) to Golgi. In this attempt, dissociation rate constants of the stable and unstable class I molecules could be obtained, and proportions of the two types of class I molecules expressed on the cell surfaces were determined. Furthermore, it was also found that IFN-γ treatment of the cells resulted in elevated expression of the class I molecules of both the stable and unstable forms.

Materials and Methods

Cell lines and antibodies

T1 and T2 cells were kindly provided by Dr. Young Yang (The Scripps Research Institute, La Jolla, USA). T2 cells have been derived from T1 cells after the spontaneous loss of CEM-derived chromosome 6, and express cell surface HLA-A2 at 30 to 50% of the level of T1 cells (Hoskin *et al.*, 1990). The hybridoma BB7.2 that produces a monoclonal antibody that binds a conformational determinant of HLA-A2 molecules, and Y-3 that produces a monoclonal antibody reactive with a conformational determinant of H-2K^b molecules were purchased from ATCC (Rockville, USA). Concentrated culture supernatant of the hybridoma was used for detecting the class I molecules expressed on the cell surface.

Synthetic peptide

The synthetic peptide WLSLLVPFV, designated as HBs 346-354, which comprises human hepatitis B virus surface antigen, was purchased from Cambridge Research Biochemicals (Cheshire, UK). The peptide was subjected to HPLC on a C_{18} reverse-phase column and eluted as a single major peak (>90%). It was solubilized in dimethylsulfoxide and diluted with PBS.

Brefeldin A, β2-microglobulin and interferon-γ

Brefeldin A purchased from Sigma Chem. Co. (St. Louis, USA) was solubilized in absolute methanol, and stored at −70℃ until used. Human recombinant interferon (IFN)-γ was kindly donated by Lucky Biotech Research Institute (Taejon, Korea).

Flow cytometric analysis of HLA-A2 molecules on T1 and T2 cells

Cells (1×10^6) were washed twice with PBS and incubated with 100 μl of BB7.2 (or Y-3 for murine cells, RMA and RMA-S) hybridoma culture supernatant or diluted normal mouse serum as a negative control for 40 min on ice. After washing three times with PBS, cells were incubated with 100 μl of 1/100 dilution of FITC-conjugated goat anti-mouse immunoglobulin (Sigma Chemical Co.) for 40 min on ice, washed twice with PBS and analyzed on a flow cytometer (Model FACScan, Becton Dickinson, Mountain View, USA).

Treatment of cells with exogenous peptide or with brefeldin A

After washing twice with serum-free RPMI, cells were incubated with or without 10 μg/ml of HBs 346-354 peptide at 37℃ for 3 h. The cells were subsequently removed from the culture, and washed three times with RPMI. If necessary, the cells were treated with 30 μg/ml of brefeldin A in culture medium for the indicated time period.

Results

T1 and T2 cells express MHC class I molecules with differential stability

It has been known that the class I MHC molecules which are empty or bound by low affinity peptides are relatively unstable compared with the class I molecules bound with optimal high affinity peptides. As demonstrated earlier (Hoskin and Bevan, 1990; Luescher *et al.*, 1992), T2 cell line showed partially decreased expression of HLA-A2 such that the expression level reached less than a half of that in T1 cells (Fig. 1A). Since it has been reported that T2 cells express the stable form of class I molecules as well as the unstable ones (Cerundolo *et al.*, 1990), attempts were made to differentiate the stability of the class I molecules on T1 and T2 cells. Thus determined was the expression level of surface class I molecules after transport of newly-assembled class I molecules from ER to Golgi was blocked by BFA treatment. The decreased expression on T2 cells was evident after treatment with BFA for 3 h such that the expression level reached only 45% that of the untreated cells, whereas the expression of class I molecules on T1 remained high even after BFA treatment (Fig. 1A). In contrast, in RMA-S, a murine cell line that is also defective in TAP gene function, K^b molecules disappeared almost completely when the cells were treated with BFA, whereas the class I expression on RMA cells that are normal in TAP gene function remained high after BFA treatment for 3 h like in BFA-treated T1 cells (Fig. 1B).

In the presence of BFA, a time-dependent decrease of fluorescence would reflect the spontaneous dissocia-

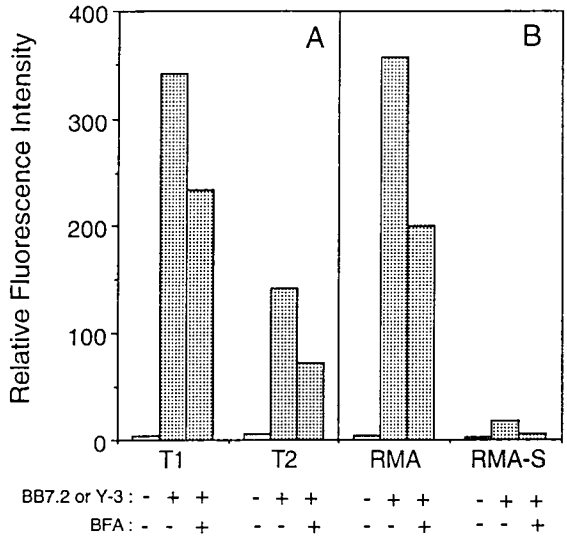

Fig. 1. Decrease of HLA-A2 expression after brefeldin A treatment. Cells were treated with brefeldin A (30 μg/ml) at 37℃ for 3 h before anaysis. They were incubated with anti-HLA-A2 antibody BB7.2 (A) or anti-K^b antibody Y-3 (B) on ice for 40 min, and subsequently incubated with goat anti-mouse immunoglobulin conjugated with FITC for 40 min and analyzed on a flow cytometer in order to determine the surface class I MHC expression levels represented by fluorescence intensities.

tion of the surface class I molecules, as represented by Fig. 2A and B. The pattern of dissociation of the class I molecules on the cell surface, as more evident with T2 cells when plotted in a logarithmic scale (Fig. 2C), showed that there seemed to exist two types of class I molecules with different dissociation rates. This suggests that the class I molecules on the cell surface consist of both stable and unstable forms of the class I complexes.

Kinetic analysis of dissociation of the class I molecules

Considering previous reports that described a low dissociation rate of the class I complexes and that its half-life ranged several hours to a day (Parker *et al.*, 1992; Ruppert *et al.*, 1993), the results mentioned above indicate that the class I molecules appeared to be expressed in at least two different forms in terms of stability and suggest that their relative stability on the cell surface can be calculated after the blocking of intracellular transport of class I molecules with BFA.

In an attempt to measure the relative dissociation rates of the surface class I molecules on both cell types, their expression levels were compared after treating the cells with BFA. Since it was not possible to identify the stable and unstable class I molecules separately in the measurement of the amount of the class I molecules by using anti-HLA-A2 antibody, a statistical anal-

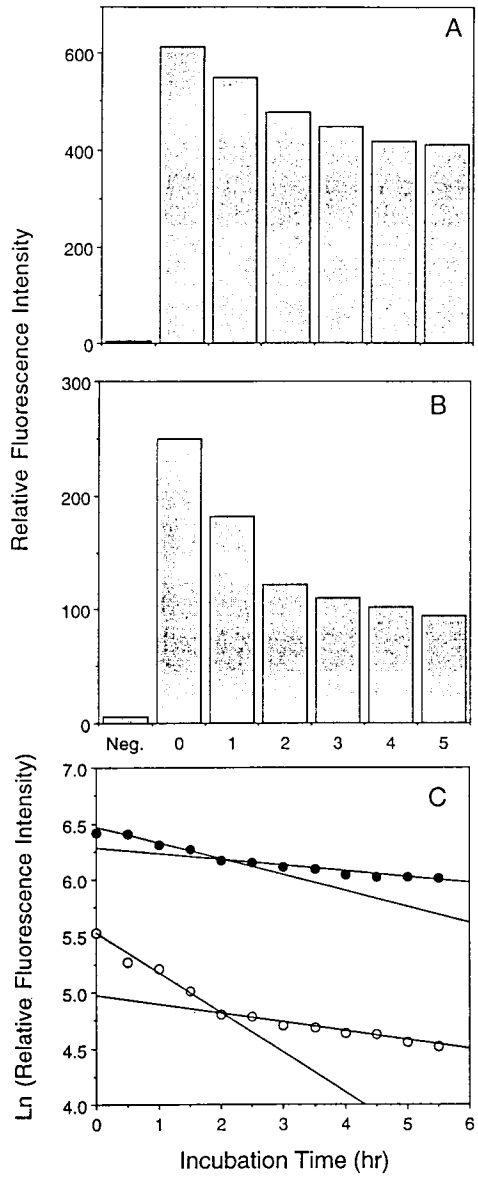

Fig. 2. Dissociation of surface HLA-A2.1 molecules on T1 and T2 cells after brefeldin A treatment. T1 (A) and T2 (B) cells were cultured with brefeldin A (30 μg/ml) at 37℃ for the indicated time period, washed, and incubated with BB7.2 for 40 min. They were subsequently incubated with goat anti-mouse Immunoglobulin conjugated with FITC for 40 min and analyzed on a flow cytometer in order to determine the surface HLA-A2 expression levels represented by fluorescence intensities. "Neg." in panels A and B stands for the fluorescence intensities obtained from the cells without any treatment. (In panel C, ●–●, T1; ○–○, T2).

ysis was needed to estimate the dissociation rate of stable and unstable class I molecules. Total amount of the HLA-A2 molecules on T1 or T2 cells can be expressed as

$$M_T = M_s + M_u \quad (1)$$

where M_T is the total amount of the undissociated class I molecules, M_s and M_u represent the amount of the

stable and unstable class I molecules, respectively. Considering the irreversible dissociation of the class I molecules, we can assume that the dissociation of the class I molecules follows the first order kinetics of

$$\frac{dM_s}{dt} = -k_sM_s \quad (2)$$

and

$$\frac{dM_u}{dt} = -k_uM_u \quad (3)$$

where t is incubation time in h, k_s and k_u are dissociation-rate constants for the stable and unstable HLA-A2 molecules, respectively, in h^{-1}. M_s and M_u at time t can then be obtained by integrating Eq. (2) and (3), with initial conditions of M_sM_{so} and $M_u=M_{uo}$ at $t=0$:

$$M_s=M_{so}\exp(-k_st) \quad (4)$$

$$M_u=M_{uo}\exp(-k_ut) \quad (5)$$

where M_{so} and M_{uo} are initial amounts of the stable and unstable class I molecules, respectively. From Eq. (1), (4) and (5), the total amount of the class I molecules can be expressed as

$$M_T=M_{so}\exp(-k_st) + M_{uo}\exp(-k_ut) \quad (6)$$

In flow cytometric analysis of HLA-A2.1 molecules, fluorescence intensity is an index of M_T, the total amount of the class I molecules. The dissociation rate constants k_s and k_u can thus be estimated from the decrease of the fluorescence intensity in the flow cytometric analysis of HLA-A2.1 molecules on T1 and T2 cells if there is no influx of new molecules to the cell surface.

The T1 and T2 cells were treated with BFA for the indicated time period, and the cells were subjected to analysis of HLA-A2 expression on the surface by flow cytometry. The resulting fluorescence intensities were plotted on a logarithmic scale against time, and they were curve-fitted with non-linear regression, as shown in Fig. 3. From the results of Fig. 3 (line A and line C), the kinetic constants k_s and k_u of Eq. (6) could be estimated by using a non-linear regression method. In this work, Sigma Plot (Jandel Scientific, San Francisco, USA) was used for the estimation of the kinetic constants. In the estimation of the constants, it was assumed that the stable or unstable HLA-A2 molecules have the same molecular structures, respectively, regardless of the cell type the molecules are expressed on and that the dissociation rate constants do not change with cell types, either. The estimated dissociation rate constants, k_s and k_u, each of which is the same for T1 and T2 cells, turned out to be 0.076 and 0.66 h^{-1}, respectively.

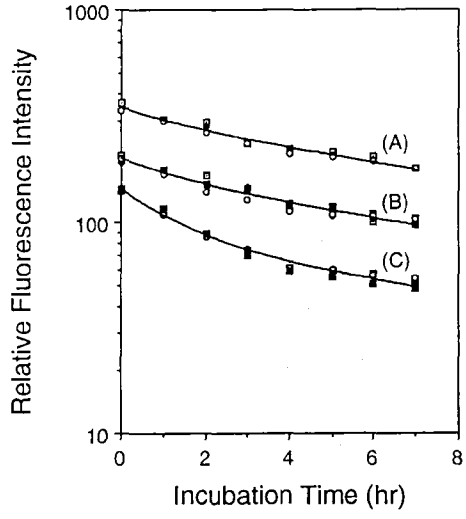

Fig. 3. Kinetics of dissociation of surface HLA-A2 molecules after brefeldin A treatment. After treatment with brefeldin A for the indicated time period, surface HLA-A2 expression on T1 (line A) or T2 (line C) was determined by using BB7.2, as described in Fig. 2. Alternatively, T2 cells were preincubated with a synthetic peptide (HBs 346-354) at a concentration of 10 μg/ml for 3 h (line B), before they were treated with brefeldin A (30 μg/ml), and analyzed for surface HLA-A2 expression as described in Fig. 2. Four independent experiments were performed for the lines A, B, and C, respectively, and all the data were used for the estimation of the kinetic parameters. The curves were generated from the equation (6), as described in the text, with parameters estimated by using a non-linear regression of the values of fluorescence intensities representing the surface expression level of HLA-A2.

With the values of k_s and k_u obtained as above, the initial amount of the stable and unstable class I molecules present on the cell surface, M_{so} and M_{uo}, was estimated from another non-linear regression of the data in Fig. 3. The estimated values of kinetic constants, M_{so} and M_{uo} are listed in Table 1. The amounts of the stable and unstable class I molecules with time could then be calculated from Eq. (6) and the data shown in Table 1. As a result of the kinetic analysis, HLA-A2 molecules expressed on the surface of T1 cells appeared to exist in stable and unstable forms at 86.4 % and 13.6%, respectively, whereas for T2 cells, it was 57.8% and 42.2%, respectively.

Elevation of class I MHC expression induced by an exogenous peptide

If T1 and T2 cells express both the stable and unstable forms of HLA-A2 molecules, it would be possible to stabilize the unstable molecules by adding exogenous peptides with high affinity for HLA-A2. It has previously been demonstrated that a nonamer peptide

Table 1. Estimated values of kinetic parameters[a]

	Stable molecule		Unstable molecule	
	M_{so}[b]	Distribution (%)	M_{uo}[b]	Distribution (%)
T1	299.1	86.4	47.3	13.6
T2	82.6	57.8	60.2	42.2
T2+peptide	163.3	81.6	36.9	18.4
Dissociation-rate constant (h^{-1})	0.076 (k_s)		0.66 (k_u)	

[a] From the data of Fig. 3, the kinetic constants k_s and k_u were estimated by using a non-linear regression method (Sigma Plot, see text for detail). M_{so} and M_{uo}, the initial expression levels of the stable and unstable HLA-A2 molecules, respectively, were calculated by executing another non-linear regression of the data with the fixed values of k_s and k_u.
[b] The values of M_{so} and M_{uo} were represented by relative fluorescence intensities.

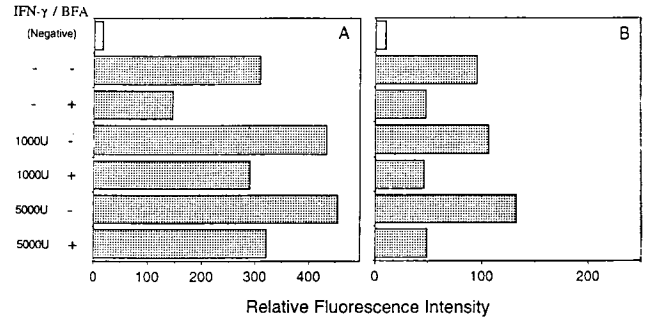

Fig. 4. Induction of surface HLA-A2 molecule expression by IFN-γ. T1 (A) or T2 (B) cells were cultured for 36 h at 37℃ in the presence or in the absence of IFN-γ. After treating with brefeldin A for 3 h, surface HLA-A2 expression was determined by using BB7.2, as described in Fig. 1. "Negative" in the vertical axis stands for the fluorescence intensities obtained from the cells without any treatment.

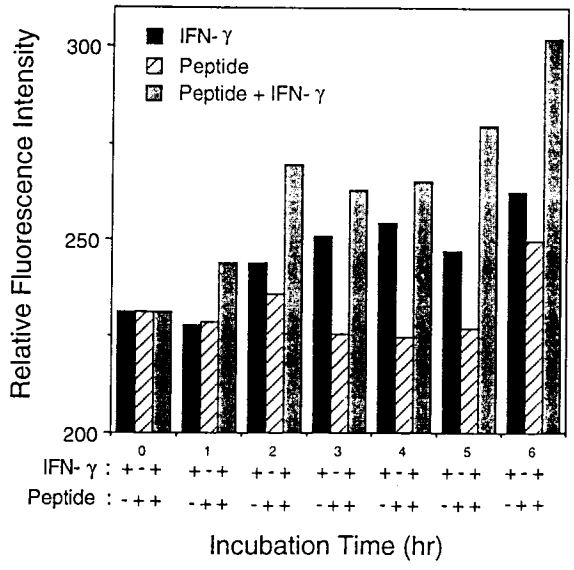

Fig. 5. Enhancement of IFN-γ-induced HLA-A2 expression on T1 cell surface by an exogenous peptide. T1 cells were cultured for the indicated time period in the presence of a peptide HBs 346-354 (10 μg/ml) and/or IFN-γ (2,500 U/ml). After washing the cells surface HLA-A2 expression levels were determined as described in Fig. 1.

HBs 346-354 binds to detergent-solubilized HLA-A2 molecules with high affinity (Elizabeth *et al.*, 1991). T2 cells were incubated with the peptide to examine whether the peptide could restore the cell surface class I expression, presumably by stabilizing the unstable class I complexes on the cell surface. As shown in Fig. 3 (line B), after adding the exogenous peptide the level of HLA-A2 expression was substantially elevated compared with that of T2 cells without peptide treatment (line C), suggesting strongly that the HLA-A2 molecules were stabilized on the peptide-treated T2 cells. The elevation of class I expression induced by peptide would probably be due to the decreased dissociation of the unstable class I. Moreover, the stabilized class I molecules appeared to dissociate at a rate similar to that observed in T1 cells (Fig. 3), and the peptide-treated T2 cells turned out to have 81.6% stable forms and 18.4% unstable forms (Table 1). These results therefore indicate that the unstable HLA-A2 molecules expressed on T2 cell surface could be stabilized after incubation with relevant peptides so that they showed dissociation kinetics very similar to that of T1 cells.

Interferon-γ treatment enhances the expression of the unstable class I MHC molecules

It is well established that IFN-γ induces the expression of class I MHC molecules in most cell types by activating an ATP-dependent peptide transporter (Giacomini *et al.*, 1988; Spies *et al.*, 1991). Addition of BFA after IFN-γ treatment for 36 h reduced the class I expression in T1 cells, as shown in Fig. 4A. The apparent dissociation rate seemed close to that of BFA-treated T1 cells without IFN-γ pretreatment, suggesting that T1 cells express both the stable and unstable form of class I after IFN-γ treatment. Since it has been reported that IFN-γ might mobilize preexisting heavy and light chains of the class I molecules to assemble and transport them to the cell surface before transcription of the class I MHC genes occur in an early stage of IFN-γ action (Vegh *et al.*, 1993), it would also be important to examine whether IFN-γ treatment of T1 cells for a short period of time induces the stable or unstable form of the class I, or both. When the cells were incubated with IFN-γ for 1 to 5 h, IFN-γ alone could significantly enhance the class I expression on T1 cells, and much higher class I expression was observed with

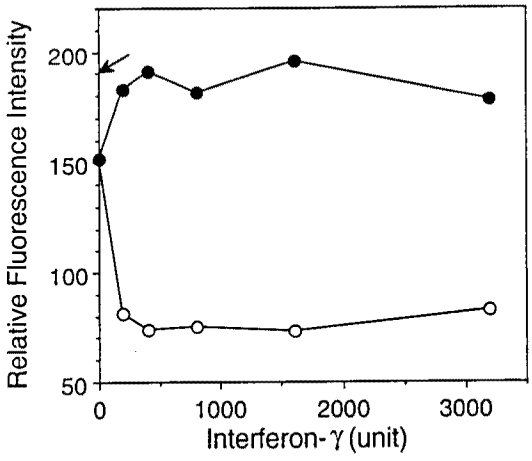

Fig. 6. Stabilization of HLA-A2 molecules on IFN-γ-treated T2 cell surface by exogenous peptides. T2 cells were cultured with IFN-γ in the presence (●–●) or in the absence (○–○) of a peptide HBs 346-354 (10 μg/ml) for 5 h. They were subsequently treated with brefeldin A (30 μg/ml) for 3 h, and surface expression levels of HLA-A2 were determined as described in Fig. 1. The arrow indicates the expression level of HLA-A2 on T2 cells that were determined after incubating the cells with the peptide followed by BFA treatment.

IFN-γ treatment in the presence of the exogenous peptide (Fig. 5). It was decided to incubate the cells with IFN-γ for less than 6 h, since it was not until 6 h after IFN-γ treatment that elevation of mRNA level for the class I molecules was detected (Végh *et al.*, 1993). The proportion of the unstable molecules among IFN-γ-induced HLA-A2 seemed to increase, since the increment of fluorescence intensity of the IFN-γ-induced and peptide-stabilized molecules seemed larger than that of the molecules treated only with peptide. This result is also consistent with an early observation that the presence of peptide counteracts a decrease of the class I level on IFN-γ treated cells (Powis *et al.*, 1993).

In T2 cells, on the other hand, only a slight increase in class I expression was observed with IFN-γ treatment for 36 h, and the expression was markedly reduced by BFA treatment to the level of that observed in BFA-treated T2 cells without IFN-γ treatment (Fig. 4B). This result implies that the class I molecules induced by IFN-γ treatment in T2 cells, if any, seemed to be mostly unstable molecules that dissociated almost completely during 5 h culture with BFA. However, 5 h exposure to IFN-γ did not seem to enhance the HLA-A2 expression, since IFN-γ treatment in the presence of HBs 346-354 peptide resulted in the expression of HLA-A2 at a level no higher than in T2 cells that were not treated with IFN-γ (Fig. 6).

Discussion

T2 cells are deficient in assembly and intracellular transport of HLA class I molecules. Not all the class I alleles are, however, affected equally such that a low level of HLA-A2, but no HLA-B5, is detected on the cell surface (Salter *et al.*, 1985; Salter and Cresswell, 1986). The class I molecules expressed on T2 cells have been reported to be similar to those synthesized by murine cell line RMA-S, which also has a defect in peptide transport due to a mutation preventing the expression of TAP-2 gene products (Cerundolo *et al.*, 1990). We explored the issue of stability of the class I molecules by culturing T1 and T2 cells in the presence of BFA which inhibits the transport of newly-assembled class I molecules to the cell surface, and by measuring the dissociation rate of the surface class I molecules. It was also reported that BFA inhibits presentation to CTLs of the endogenously processed antigens, but not exogenously added synthetic peptides (Misumi *et al.*, 1986; Nuchtern *et al.*, 1989).

We found that after BFA treatment for 3 h HLA-A2 molecules on T2 did not completely dissociate into heavy chains and β2m, while K^b molecules on RMA-S cells appeared to be almost completely depleted after BFA treatment. These results suggest that T2 cells express the stable form of class I as well as the unstable form in spite of deletion of transporter genes. This was not due to contamination or transformation of cells, because they clearly showed no expression of HLA-B5 molecules measured by anti-HLA-B5 antibody, 4D12 (data not shown). Baas *et al.* (1992) described that incubation of T2 cell lysate over 35℃ results in only a slight decrease in the amount of anti-class I (W6/32)-reactive material for HLA-A2, and argued that the empty class I molecules might partially unfold, but do not necessarily dissociate at elevated temperatures. Thus, it is very likely that T2 cells express not only unstable HLA-A2 molecules on the cell surface but also stable ones, probably occupied by peptides, which remains at a relatively high level even after BFA treatment. In the presence of appropriate binding peptide, the unstable class I molecules could be converted to the stable forms, and thus it was observed that the peptide-stabilized molecules on the cell surface did not readily dissociate even in the presence of BFA. This observation is consistent with the notion that the low level expression of the class I molecules reflects the inability of T2 cells to generate stable and functional class I molecules rather than the inability to synthesize heavy and light chains of the class I molecules. On the other hand, these results might reflect the argument that the peptide bound weakly as a result of insufficient amount of extracellular β2m available as proposed by Madrigal

et al. (1991). However, in the presence of sufficient amount of dialyzed human β2m, no significant difference was observed by us (data not shown), indicating that class I molecules on T2 cell surface were expressed in β2m-associated form.

Although it seems clear that there are two different types of class I molecules in terms of their molecular stability, it has not been certain what the ratio of the stable and unstable complexes expressed on T1 and T2 cells is. In our hands, T1 cells express approximately 86% of the stable form and approximately 14% of the unstable form, whereas T2 cells express approximately 58% and 42%, respectively. The dissociation rate constants of the stable and unstable complexes turned out to be 0.076 h^{-1} and 0.66 h^{-1}, respectively, indicating that the unstable molecules dissociate approximately 9 times as fast as the stable molecules. Although the early attempts to measure the binding of labeled peptides to purified HLA molecules have given at best occupancy of less than 1% at the end of the incubation period, such binding data are difficult to interpret and raise questions as to whether the extent of binding actually represented the peptides bound to intact HLA molecules (Chen and Cresswell, 1989). It was also reported that in intact cells 9.2% of the total accessible surface D^b molecules are able to bind peptides (Christinck *et al.*, 1991). It is not clear whether the peptide-accessible portion of the D^b molecules represents the unstable form of class I, as described in this report, or that they simply reflect exchange of peptides bound to the stable molecules. However, it is very likely that they represent the unstable class I molecules since the dissociation rate contant of the D^b molecules in the report (4×10^{-4} sec^{-1}) is close to that described in this report (0.66 h^{-1} or 1.8×10^{-4} sec^{-1}). Whether this unstable fraction described in this report is able to accept peptides by virtue of being 'empty' or being in possession of a lower affinity peptide that can be displaced by exchange remains to be determined. These kinetic analysis were performed on the assumption that the HLA-A2 molecules once dissociated do not reassociate into complete class I molecules on the cell surface in several hours and that internalization of the class I molecules during the incubation is negligible. It should also be assumed that all the HLA-A2 molecules on T1 or T2 cells are represented as either of the two forms, stable or unstable. It is likely that the stable and unstable molecules do not necessarily exist as homogeneous populations in terms of molecular stability, rather they might represent a group of molecules with certain ranges of stability and be divided into a stable and an unstable groups.

IFN-γ enhances the transcription and influences several post-transcriptional events of the synthesis of the class I molecules (Klar and Hämerling, 1989). In our experiments IFN-γ treatment induced the expression of stably assembled class I molecules as well as unstable molecules that readily dissociate with BFA treatment. For T1 cells treatment with IFN-γ appeared to enhance expression of both the stable and unstable forms of the HLA-A2 molecules. This result might reflect that the size of the pool of presentable peptides available to class I molecules is limited, and thus resulted in the shortage of peptide supply to meet increased level of the class I molecules during stimulation by IFN-γ.

In T2 cells, on the other hand, a long-term IFN-γ treatment resulted in a slight enhancement in class I expression, and the induced class I molecules, if any, seemed to be mainly unstable molecules which dissociate rapidly when treated with BFA. The result would reflect again the limited supply of the binding peptide in T2 cells did not meet the demand of peptides by increased number of class I molecules. It was also observed that a brief exposure to IFN-γ in the presence of relevant peptide did not show any enhanced class I expression level after BFA treatment, suggesting that IFN-γ is not able to mobilize preexisting heavy and light chains of the class I molecules to assemble into complete molecules in T2 cells (Giacomini *et al.*, 1988). This may also reflect the possibility that the deleted genomic segment(s) in T2 cells that includes TAP genes also involves the gene(s) that is required for the action of IFN-γ. It has similarly been reported that BFA treatment of a class I-deficient cell line CMT 64.5, even in the presence of IFN-γ, abolished its presentation of viral antigens to a CTL clone (Sibille *et al.*, 1992).

The observation that HLA-A2 molecules were expressed in a significantly high level on T2 cells even after treatment with IFN-γ along with BFA raises the possibility that class I molecules in T2 might be generated from an alternative pathway other than the BFA-sensitive pathway, or machinery that loads peptides of a different intracellular source. It has been reported that, in contrast to classical class I-mediated antigen presentation, the presentation of Sendai virus antigen in K^b-tranfected T2 cells is BFA-insensitive, raising a possibility of the presence of an alternative pathway for MHC class I-mediated antigen presentation in T2 cells (Zhou *et al.*, 1993). It was similarly speculated that the system that delivers antigenic peptides from the cytosol to the ER in RMA-S still exists and partially functions, and the class I molecules in cell lysates are occupied by peptides to some extent (Esquivel *et al.*, 1992; Hosken *et al.*, 1992). It is also possible, as postulated recently, that a mechanism for retaining incompletely assembled class I molecules works differently in T1 and T2 cells

(Ou *et al.*, 1993). Regardless of the mechanisms involved, it is evident that there is always a small proportion of unstable class I molecules and the degree of expression of these molecules may be dependent on the MHC allele and cell type used.

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