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Induction of assembly of MHC class I heavy chains with $\beta_2$microglobulin by interferon-γ

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Assembly of histocompatibilty class I heavy chains with $\beta_2$microglobulin (β_2m) is known to be necessary for cell surface expression. Studies on the H-2 class I deficient but interferon-γ (IFN-γ) inducible fibrosarcoma BC2 and the lung carcinoma CMT 64.5 showed that after transfection with allogeneic H-2 class I genes the class I proteins are expressed, but only intracellularly and not on the cell surface. In spite of the presence of β_2m in the cells no association of the transfected class I chain with β_2m was observed. However, stimulation with IFN-γ induced assembly and subsequent surface expression. These findings show that the assembly of class I heavy chains with β_2m is not a spontaneous event but appears to be regulated by cellular mechanisms the nature of which is still unknown.

Key words: $\beta_2$microglobulin/class I heavy chains/IFN-γ/ regulation of assembly

Introduction

The major histocompatibility complex (MHC) encodes a group of highly polymorphic cell surface antigens of M_r ~45 000 that serve as recognition structures in the immune system. For example, cytotoxic T lymphocytes (CTL) recognize cells infected with virus or altered by neoplastic transformation only in conjunction with MHC class I antigens (reviewed by Zinkernagel and Doherty, 1979 and Hämmerling *et al.*, 1987). The classical class I antigens, HLA-A,B,C in the human and H-2-K,D,L in the mouse, are expressed on most nucleated cells although in differing amounts. In most normal tissues or tumour lines with low class I expression, a strong enhancement can be achieved by treatment with IFN-γ (e.g. Wong *et al.*, 1984; Momburg *et al.*, 1986). Work with tumour lines has suggested that IFN-γ stimulates transcription of class I genes (Fellous *et al.*, 1982; Morello *et al.*, 1982; Eager *et al.*, 1985). This effect appears to be mediated via an IFN consensus sequence adjacent to an enhancer sequence in the 5′ end of class I genes (Friedman and Stark, 1985; Israel *et al.*, 1986; Kimura *et al.*, 1986).

On the cell surface class I antigens are non-covalently associated with a light chain of M_r 12 000, designated $\beta_2$microglobulin (β_2m) (Rask *et al.*, 1974). β_2m has been found to be necessary for cell surface expression of the class I heavy chains (Arce-Gomez *et al.*, 1978; Ploegh *et al.*, 1979). Mutants with a defect in β_2m expression, such as the Daudi cell line, accumulate in the endoplasmic reticulum the HLA heavy chain in the high mannose form which is not further processed (Ploegh *et al.*, 1979). The murine D^b molecule seems to represent an exception to this rule reaching the surface in the absence of β_2m (Allen *et al.*, 1986). However, in this situation the conformation of D^b is drastically altered because only an antibody against the α_3 domain is still reactive but none of the many antibodies against the α_1 and α_2 domains (Allen *et al.*, 1986). Numerous studies on the biosynthesis of class I antigens suggested the following scheme of events of which details still need to be clarified. Expression of class I and β_2m genes can be independently regulated (Morello *et al.*, 1985). During or shortly after translation the class I heavy chain is inserted into the endoplasmic reticulum. This event is followed by glycosylation of the heavy chain and association with β_2m (Dobberstein *et al.*, 1979; Owen *et al.*, 1980). These heavy and light chain dimers appear within minutes after their synthesis (Krangel *et al.*, 1979). The assembled high mannose form is then transported to the Golgi apparatus and processed resulting in the terminally glycosylated form (Owen *et al.*, 1980; Dunphy *et al.*, 1985; Williams *et al.*, 1985). Only this mature form will be transported to the plasma membrane and expressed on the cell surface (Van Le and Doyle, 1985).

One of the crucial steps in this pathway is the assembly of the class I heavy and β_2m light chains. So far it is not known if the assembly is a spontaneous process or if an active cellular mechanism is required.

The present report provides evidence for the latter possibility. We analysed H-2 class I deficient cell lines which respond to IFN-γ treatment. We observed that transfected H-2 and HLA genes were only expressed in the cell and not on the cell surface. In spite of the presence of large amounts of β_2m in these cells, the class I heavy chains did not associate with β_2m. However, assembly and subsequent cell surface expression could be induced with IFN-γ.

Results

Induction of H-2 expression with IFN-γ in H-2 deficient tumour lines

Two different murine tumour lines with low H-2 class I expression; the spontaneous lung carcinoma CMT 64.5 and the methylcholanthrene A-induced fibrosarcoma BC2, were investigated for their molecular defect. They are both derived from C57Bl/6 mice which are of the $H\text{-}2^b$ haplotype. Analysis with monoclonal anti-H-2 class I antibodies (mAb) by cellular radioimmunoassay (see Table IA) and by cytofluorometry (not shown) revealed that the cells did not express K^b antigens on the surface and expressed only marginal amounts of D^b antigens. However, after *in vitro* treatment with IFN-γ a strong enhancement of K^b and D^b expression was obtained. This effect was transient and persisted for 3–5 days after removal of IFN-γ as has been described previously for other cell types (e.g. Collins *et al.*, 1984).

Table I. Cell surface expression of H-2 class I antigen

Cell line	Transfected gene	IFN-γ	Anti H-2 antibody (c.p.m. bound)				
			K^b	D^b	K^k	K^d	D^k
A							
BC2	–	–	1348	1702	1010	–	–
		+	*8950*	*27 316*	968	–	–
CMT 64.5	–	–	420	1155	1128	852	1188
		+	*15 140*	*31 644*	872	970	1148
B							
BC2.K^k-1	K^k	–	644	872	1022	410	–
		+	*7195*	*10 977*	*28 856*	890	–
CMT.cK^d-1	K^dcDNA	–	1205	1967	1616	1975	432
		+	*7223*	*28 555*	1612	*8060*	472
CMT.SVK^k-1	pSVTK-K^k	–	480	1124	1964	468	–
		+	*15 292*	*17 340*	*24 224*	492	–
CMT.D^k-1	D^k	–	952	1580	1136	–	1044
		+	*8108*	*20 148*	788	–	*16 924*
BC2.K^k.β_2m-1	K^k+β_2m	–	134	278	148	170	–
		+	*4336*	*5500*	*21 492*	216	–

(**A**) Expression of endogenous H-2 class I antigens. BC2 and CMT 64.5-cells were cultured for 2 days with and without 20 units/ml IFN-γ. H-2 class I expression was then screened in a cellular radioimmunoassay with the monoclonal antibodies described in Materials and methods.
(**B**) Expression of transfected H-2 class I genes. BC2 and CMT 64.5-cells were transfected with the various H-2 class I genes as indicated in Materials and methods and their H-2 class I expression was measured before and after IFN γ-treatment. Numbers represent the mean value of triplicate wells. Unspecific binding was ~500 to 1000 c.p.m. Positive values are in italics.

Immunoprecipitation studies with monoclonal anti-K^b antibodies revealed that K^b proteins could only be found after stimulation with IFN-γ (see Figure 1B, lane 2 and 4). Since long term labelling was performed both mature and immature forms of the K^b protein were present. Of the two bands in the 46-kd range the lower one represents the immature form with high mannose side chains and the higher band represents the mature H-2 antigen containing complex type sugars. It is well documented that processing and addition of complex type sugars leads to a shift towards higher mol. wt (e.g. Williams *et al.*, 1985). The 12 kd band represents β_2m which is associated with the class I antigens and therefore coprecipitated with anti-class I antibodies.

IFN-γ strongly enhanced the synthesis of K^b-specific mRNA as determined in dot blot analysis with a K^b-specific oligonucleotide probe (see Figure 1A, lanes 2 and 4). Hybridization with a β-actin probe served as an internal control. These data suggest that the defect of the tumour lines is located on the level of H-2 class I gene transcription and/or RNA stability. Similar observations have been made previously with other IFN-γ-inducible tumour lines (Fellous *et al.*, 1982; Morello *et al.*, 1982; Schrier *et al.*, 1983).

Expression of transfected H-2 class I genes

In order of see if H-2 class I genes transfected into these tumour lines are under the same control as the endogenous H-2 class I genes, the tumour lines were transfected with various class I genes. The use of allogeneic class I genes allowed us to distinguish between expression of the endogenous H-2^b genes and the transfected genes. When the transfectants were screened with anti-H-2 class I antibodies in a cellular radiommunoassay, cell surface expression of the products of transfected H-2 class I genes was only observed after treatment of the cells with IFN-γ. Comparable results were obtained with genomic clones of the murine K^k, K^d, D^k and the human HLA-Cw3 gene, as well as with a K^k gene controlled by a TK promoter and a SV40 enhancer (pSVTK-K^k), with a K^d-specific cDNA under control of a SV40 promoter (K^dcDNA), and in cells transfected with both the K^k and β_2m genes. Some representative examples are shown in Table IB.

Because surface expression was not obtained without IFN-γ-treatment, expression of the transfected H-2 class I genes was investigated on the RNA and protein level. In the following sections results obtained with BC2 cells transfected with the K^k gene and CMT 64.5 cells transfected with the K^d cDNA are presented. These data are also representative for the other H-2 transfectants.

Intracellular expression of transfected H-2 class I genes

Dot blot analysis of cytoplasmic RNA with a K^k-specific oligonucleotide probe showed that the transfected K^k gene was clearly expressed in the absence of IFN-γ, and that expression could be enhanced with IFN-γ (Figure 2A, lanes 1 and 2). Immunoprecipitation analysis of K^k or K^d transfected cells with anti-H-2 class I antibodies showed that intracellularly, considerable amounts of transfected K^k or K^d heavy chains were present (Figure 2B, lanes 1 and 3).

Two aspects of the immunoprecipitated class I heavy chain encoded by transfected genes have to be emphasized. First, without stimulation with IFN-γ, the heavy chains were present only in the immature high mannose form. This will be demonstrated in the following section. Second, no β_2m was coprecipitated suggesting that these heavy chains were not associated with the β_2m light chain (Figure 2B, lanes 1 and 3). However, after IFN-γ treatment the transfected H-2 class I antigens were clearly associated with β_2m as indicated by the coprecipitation of β_2m with the anti-H-2 class I antibodies (Figure 2B, lanes 2 and 4). This obser-

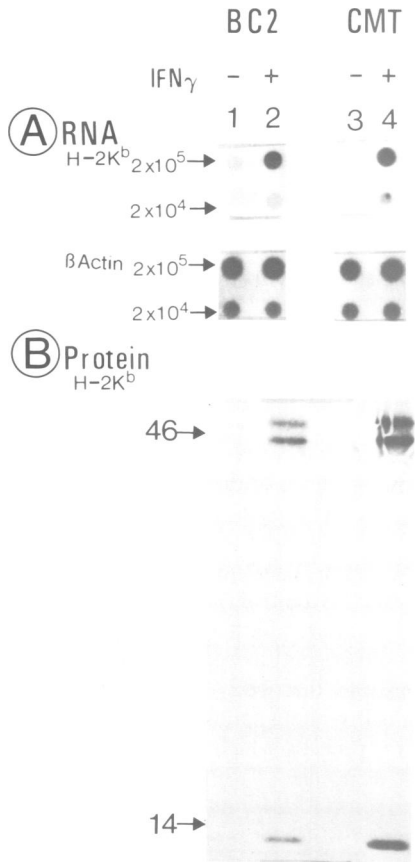

Fig. 1. RNA and protein of endogenous K^b antigen. (**A**) Dot blot analysis of intracellular K^b RNA. RNA-containing lysates prepared from 2×10^5 or 2×10^4 untreated and IFN-γ-treated BC2 and CMT 64.5 cells were dotted onto nitrocellulose and hybridized with the K^b oligonucleotide (upper panel, lanes 1–4) as well as with an actin probe as a control for the amount of RNA loaded (lower panel, lanes 1–4). (**B**) Immunoprecipitation of the intrinsic K^b protein. Untreated and IFN-γ-treated BC2- and CMT 64.5 cells were metabolically labelled for 2 h before precipitation with the anti-K^b monoclonal antibody K10-56 (lanes 1–4). Separation of precipitates was performed on 10–15% SDS–PAGE. Positions of mol. wt markers (46 and 14 kd) are indicated. All lanes were derived from the same gel.

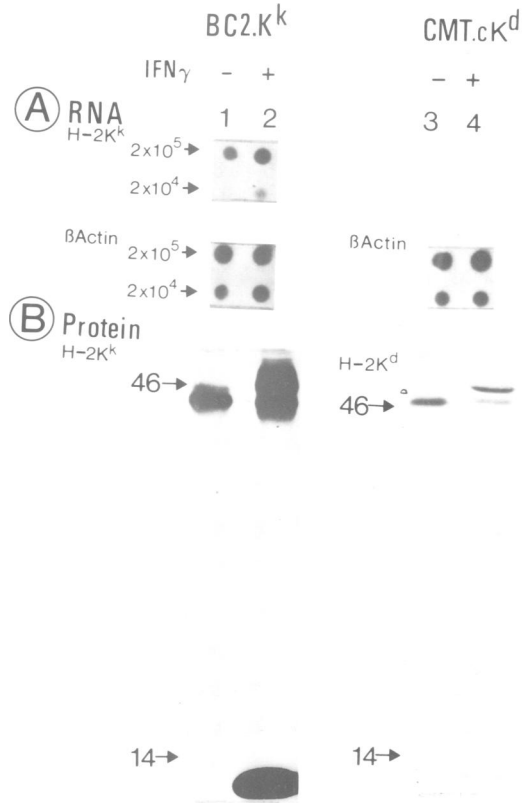

Fig. 2. RNA and protein of transfected K^k and K^d antigens. (**A**) RNA dot blot analysis of K^k-transfected BC2. K^k-specific RNA was detected with a K^k-specific oligonucleotide (upper panel, lanes 1 and 2). Control experiments with cell lines of the H-2^b and H-2^k haplotype showed that the oligonucleotide binds specifically to K^kRNA (data not shown). Hybridization with an actin probe served as a control for the amount of RNA loaded (lower panel, lanes 1–4). The K^d specific RNA of the CMT.cK^d transfectant was not analysed. (**B**) Immunoprecipitation of transfected K^k and K^d proteins. The BC2.K^k-1 and CMT.cK^d-1 transfectants (untreated or treated with IFN-γ) were metabolically labelled for 2 h. Immunoprecipitations were performed with the monoclonal antibodies H100-5 (anti-K^k, lanes 1 and 2) and K9-18 (anti-K^d, lanes 3 and 4). These antibodies do not crossreact with H-2^b antigens. The immunoprecipitates of the BC2.K^k-1 and the CMT.cK^d-1 transfectants were analysed on a separate gel as indicated by the different positions of the mol. wt markers.

vation was consistent with the surface expression obtained after IFN-γ treatment.

Expression of the transfected H-2 class I gene as the immature high mannose form

As mentioned above it is evident from Figure 2B that the free H-2 class I antigen immunoprecipitated from unstimulated cells had a lower apparent mol. wt than the additional H-2 class I form immunoprecipitated after IFN-γ treatment. Endoglycosidase H (endo-H) is an enzyme which distinguishes between the complex type cell surface form of glycoproteins and the immature high mannose form. Only the latter which has a lower mol. wt is cleaved by endo-H.

It is shown in Figure 3 (lanes 2 and 4) that endo-H digested the free heavy chain of 5 min pulse or 2 h long term labelled cells suggesting that this band corresponds to the immature high mannose form. In contrast, the upper H-2 class I band obtained after stimulation with IFN-γ was resistant to endo-H treatment suggesting that this band represents the processed and terminally glycosylated form of class I antigens (Figure 3, lanes 7 and 8). The lower H-2 class I band obtained from the IFN-γ stimulated and long term labelled BC2.K^k-1 transfectant (Figure 3, lane 7) was also endo-H sensitive (Figure 3, lane 8) and therefore corresponds to the high mannose form which has not yet been processed. Similar results were obtained with CMT 64.5 cells transfected with the K^d specific cDNA (not shown). It should be noted here that in general, the high mannose form of the class I heavy chain is able to complex with β_2m (Williams *et al.*, 1985). This is also shown in Figure 3 (lanes 5 and 6) where the endo-H-sensitive high mannose form of the K^k antigen obtained after pulse labelling of IFN-γ treated cells was associated with β_2m.

Induction of H-2 class I and β_2m assembly with IFN-γ

The lack of β_2m in H-2 class I immunoprecipitates obtained from unstimulated transfectants could have been explained by the absence of β_2m from the cells. Therefore an RNA dot blot analysis with a β_2m probe and immunoprecipitation

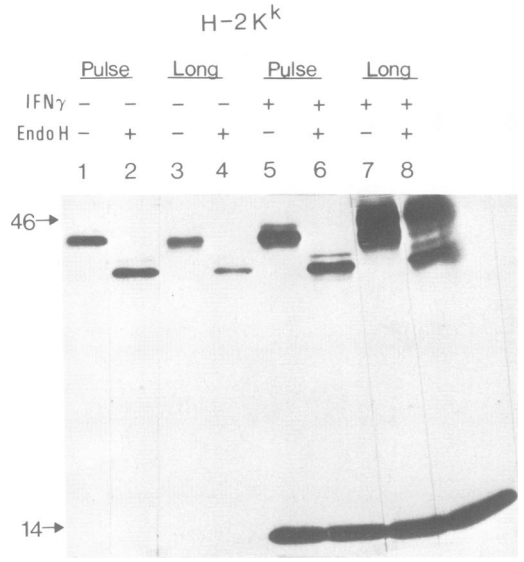

Fig. 3. Endoglycosidase H treatment of K^k molecules. IFN-γ-treated and untreated BC2.K^k-1 cells were long term (2 h) and pulse-labelled (5 min) before precipitation with the anti-K^k monoclonal antibody H100-5. Precipitates were divided into two parts and either submitted to endo-H digestion or to mock digestion. Separation of precipitates was performed on 10–15% SDS–PAGE. All lanes were derived from the same gel.

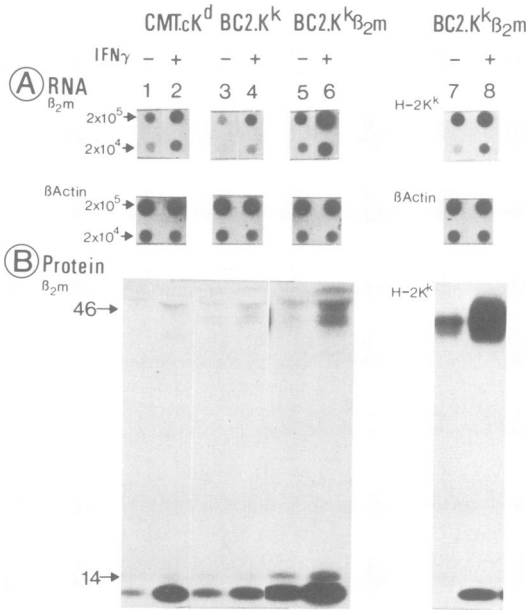

Fig. 4. β_2Microglobulin and K^k. (**A**) Dot blot analysis of intracellular $\beta_2$microglobulin RNA and K^k RNA. β_2Microglobulin-specific cytoplasmic RNA from untreated and IFN-γ-treated CMT.cK^d-1, BC2.K^k-1 and BC2.K^k.β_2m-1 transfectants was detected by hybridization with a $\beta_2$microglobulin cDNA fragment (upper panel, lanes 1–6). K^k-specific RNA in the BC2.K^k.β_2m-1 transfectant was detected by hybridization with the K^k-oligonucleotide (upper panel, lanes 7 and 8). BC2.K^k.β_2m-1 is BC2 transfected with both the K^k and β_2m genes. The hybridization with an actin probe served as an internal control (lower panel, lanes 1–8). (**B**) Immunoprecipitation of the $\beta_2$microglobulin and the K^k molecules. Cells were labelled for 2 h. Free $\beta_2$microglobulin molecules were precipitated with a rabbit anti-mouse $\beta_2$microglobulin serum (lanes 1–6). K^k molecules were precipitated with the monoclonal antibody H100-5 (lanes 7 and 8). All lanes were derived from the same gel.

studies with rabbit anti-mouse β_2m serum were performed. The rabbit serum precipitates predominantly free β_2m chains. The data presented in Figure 4 show that the transfectants BC2.K^k-1 and CMT.cK^d-1 already expressed considerable amounts of β_2m message and protein in the absence of stimulation with IFN-γ (lanes 1 and 3). As expected, the expression was enhanced after stimulation with IFN-γ (lanes 2 and 4).

To eliminate the possibility that the amount of β_2m observed in the absence of IFN-γ was too low for effective association with the class I heavy chain, the BC2 cells were not only transfected with the K^k gene but also with the murine β_2m gene. These transfectants (BC2.K^k.β_2m) expressed large amounts of β_2m RNA and protein in the absence of IFN-γ, as shown in the dot blot and immunoprecipitation studies presented in Figure 4, lane 5. However, in spite of the presence of these large amounts of β_2m light chains, only free H-2 class I heavy chains were immunoprecipitated by the anti-K^k antibody in the absence of IFN-γ (Figure 4B, lane 7) and no surface expression was found (see Table IB). As before, assembled H-2 and β_2m were only observed after stimulation with IFN-γ (Figure 4B, lane 8) with subsequent surface expression (Table IB). These data demonstrate that H-2 class I and β_2m chains can coexist in a cell without spontaneous assembly and that the assembly is a process which can be stimulated.

Reactivity of anti-K^k monoclonal antibodies with free and assembled K^k chains

Since assembly with β_2m is likely to change the conformation of the heavy chain (Krangel *et al.*, 1979) we expected that not all K^k-specific mAb would bind the free K^k heavy chain. The immunoprecipitation data presented in lanes 1–3 of Figure 5 show that only mAb H100-5 precipitated the free K^k chain from unstimulated BC2.K^k.β_2m-1 cells, whereas no or only weak precipitation was obtained with the other anti-K^k antibodies H100-27 and H116-22 which are directed against different K^k epitopes (Lemke *et al.*, 1979). In contrast, all three mAb precipitated K^k chains associated with β_2m from IFN-γ treated cells (lanes 4–6). These data further support the notion that in unstimulated cells, the K^k molecule is indeed present as a free heavy chain.

Discussion

The main observation of the present report is that in the two H-2 class I deficient tumour cell lines investigated here, the transfected H-2 class I antigens exist as free heavy chains without association with the β_2m chains also present in the cells. However, assembly can be obtained by treatment of the cells with IFN-γ. Due to the lack of assembly in unstimulated cells the immature high mannose form accumulates in the endoplasmic reticulum and is not transported to the Golgi where terminal glycosylation takes place (Dunphy *et al.*, 1985). Consequently, no cell surface expression is obtained. Double transfection studies with β_2m showed that the amount of β_2m was not a limiting factor. Likewise the use of a K^d cDNA driven by a SV40 promoter which is not responsive to IFN-γ suggests that the amount of the H-2 class I heavy chains present in the absence of IFN-γ is not limiting. Therefore the findings indicate that the association of MHC class I heavy chains and β_2m does

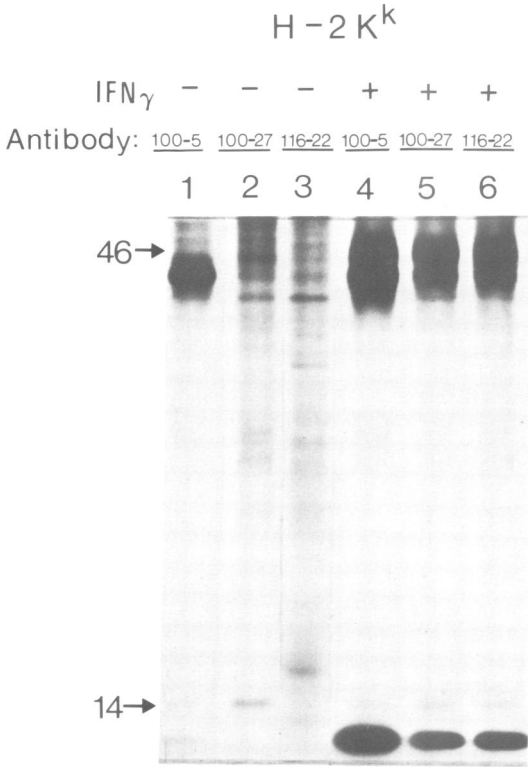

Fig. 5. Immunoprecipitation of K^k molecules with different anti-K^k monoclonal antibodies. BC2.K^k.β_2m-1 cells cultured with and without IFN-γ were metabolically labelled for 2 h. Lysates were divided into three aliquots and precipitates were made with the three different monoclonal anti-K^k antibodies H100-5, H100-27 and H116-22. All lanes were derived from the same gel.

not happen spontaneously but is a process which can be regulated. Recently, a class II deletion mutant of a human B cell line was described expressing free HLA-A2 and B5 heavy chains without association with β_2m in the cytoplasm (De Mars *et al.*, 1985; Salter and Cresswell, 1986). In somatic hybrids with HLA positive cells, assembly of the HLA-A2 and B5 heavy chains with β_2m was observed (Salter *et al.*, 1985). However, in contrast to the present study no induction of assembly by IFN-γ was reported and it is unclear whether the molecular defects in the human B cell variant are comparable to the murine tumour cells described here.

As yet very little is known about the mechanisms required for assembly of class I chains and β_2m. Two potential models can be considered. For example, the tumour cells could lack one or more positive factors which facilitate assembly and which can be induced by IFN-γ. Alternatively the cells may contain blocking factors which prevent assembly and which can be downregulated or neutralized via stimulation with IFN-γ.

Preliminary data suggest that the introduction of surface expression of the transfected H-2 antigens is a slow process requiring ~48 h treatment with IFN-γ for maximal induction (data not shown). This observation argues in favour of the suggestion that IFN-γ induces factors involved in the assembly. Experiments designed to block the induction of these hypothetical factors using cycloheximide are difficult to perform since cycloheximide also blocks the synthesis of the transfected H-2 antigens.

For several multichain complexes such as the immunoglobulins (Haas and Wabl, 1983) or the trimeric complex formed by the influenza haemagglutinin (Gething *et al.*, 1986), a 70 kd protein has been postulated to be involved in assembly. However the precise role of this 70 kd protein (designated immunoglobulin heavy chain binding protein 'BIP', or heat-shock protein 'hsp 70') is still unclear. It has been speculated that it binds to free glycoprotein chains in order to prevent malfolding and aggregation (Munro and Pelham, 1986). In the case of the immunoglobulins and the influenza haemagglutinin, BIP is released when the respective multichain complexes are formed. In our immunoprecipitation studies with anti H-2 class I antibodies we have not found such BIP-like proteins associated with the free H-2 class I chain, neither before nor after stimulation with IFN-γ. Also an anti-BIP antibody (generously provided by Drs Henderson and J.F.Kearney, Alabama) precipitated comparable amounts of BIP from unstimulated and IFN-γ treated transfectants but no coprecipitation of H-2 class I chains was observed (data not shown). Likewise, the same precipitation studies failed to reveal the presence of blocking factors associated with a free H-2 class I chain, but it cannot be excluded that the anti-H-2 class I antibodies used for immunoprecipitation dissociated the putative blocking proteins. Proteins which prevent class I surface expression have been described in certain adenovirus infections (Pääbo *et al.*, 1986). However, in the case studied in greater detail the viral E3/19K protein did not inhibit the assembly but prevented the release of the assembled class I-β_2m complex from the endoplasmic reticulum (Burgert *et al.*, 1987).

It appears that the two cell lines described here have at least two distinct defects in the biosynthesis of H-2 class I antigens, namely on the level of RNA transcription or stability, and on the level of the assembly of the class I heavy chain and β_2m. Both defects can be overcome by activation with IFN-γ. It is possible that the tumour cells represent a particular stage of differentiation which differs from mature cells in several features. Not all class I-deficient and IFN-γ inducible tumour lines seem to have the additional defect on the level of assembly because other investigators have reported constitutive cell surface expression of H-2 class I genes transfected into IFN-γ inducible cell lines (Tanaka *et al.*, 1986; Bahler *et al.*, 1988). The inducible cells described here appear to be a suitable model for the study of the cellular mechanism and factors involved in assembly. In addition, it will be of interest to determine whether the defect in assembly is restricted to class I antigens or whether assembly of other proteins such as influenza haemagglutinin or class II antigens is also affected.

Materials and methods

Cell lines, H-2 genes and transfection

The tumour cell line BC2 is a clone of a fibrosarcoma induced in a C57B16 mouse with methylcholanthrene A (Hämmerling *et al.*, 1987). CMT 64.5 is a spontaneous lung carcinoma of C57Bl6 origin (Franks *et al.*, 1976). The cells were maintained in RPMI 1640 medium supplemented with 7% FCS.

The following H-2 class I genes were used: K^k (27-2-86) (Arnold *et al.*, 1984), K^d (191-6) (Kvist *et al.*, 1983), D^k (pDK17) (Stephan *et al.*, 1986), pSVTK.K^k is a K^k gene under the control of the TK-promoter and SV40 enhancer (constructed and provided by Dr W.Pülm), the K^d specific cDNA is under the control of the SV40 promoter region (pH-2^d SV 33, designated K^dcDNA, generously provided by Dr M.Cochet) (Cochet *et al.*, 1986),

the plasmid pBM 184 neo contains the β_2m gene (allele b) plus the *neo*^{r} gene (kindly provided by Dr P.J.Robinson).

Cotransfection with the *neo*^{r} gene (pAG60) (Colbere-Garapin *et al.*, 1981) was performed as described (Wigler *et al.*, 1979) using the calcium phosphate technique. Selection of transfectants was performed in medium containing 0.6 mg/ml geneticin-G418 sulphate.

Treatment with interferon-γ

The recombinant murine interferon-γ (IFN-γ) derived from *Escherichia coli* was a kind gift from Dr Swetly (Boehringer, Wien).

The cells were cultured to a monolayer of 30–50% density. IFN-γ was added to a final concentration of 10–20 U/ml, and cells were used after an additional 48 h of culture.

Determination of H-2 expression

Surface H-2 class I antigens were determined with a cellular radioimmunoassay as described (Lemke *et al.*, 1978). Briefly, 5×10^5 cells were incubated with anti-H-2 class I antibodies followed by ^{125}I-labelled protein A.

The following anti-H-2 class I monoclonal antibodies (mAb) were used: anti K^k H100-5, H100-27, H116-22; anti D^b B22-249 (Lemke *et al.*, 1979); anti K^b K10-56 (Hämmerling *et al.*, 1982); anti K^d K9-18 (Arnold *et al.*, 1985); anti K^k 15-5-5s (Sachs *et al.*, 1981).

Metabolic labelling and immunoprecipitation of H-2 class I antigens and free $\beta_2$microglobulin

Free $\beta_2$microglobulin was detected with a rabbit anti-mouse $\beta_2$microglobulin serum kindly provided by P.J.Robinson (Robinson *et al.*, 1981). Immunoprecipitation was performed according to Dobberstein *et al.*, 1979 with some modifications. Briefly $3-4 \times 10^6$ tumour cells were long term labelled (2 h) or pulse labelled (5 min) at 37°C in the presence of 100 μCi [^{35}S]methionine and lysed in ice cold Hepes buffer containing 1% Triton X-100. After pre-absorption with protein-A Sepharose, H-2 class I proteins and free $\beta_2$microglobulin were precipitated using 300 μl culture supernatant of anti-H-2 class I antibody producing hybridomas or 3 μl of the anti-β_2m serum followed by protein-A Sepharose. The precipitates were analysed by 10–15% SDS–PAGE (Maizel, 1969). The gels were processed with 2.5-diphenyloxazole, dried and exposed using Kodak XAR-5 film (Bonner and Laskey, 1974).

Endoglycosidase H treatment

Immunoprecipitates were incubated with 0.003 U endo β.*N*-acetylglucosaminidase (endo-H, Boehringer) for 15 min at 37°C as described by Owen *et al.* (1980). A mock digestion without endo-H was performed in parallel.

RNA dot blots and probes

$1-10 \times 10^6$ cells were lysed according to the method of White and Bancroft, 1982. Equivalents of 2×10^5 and 2×10^4 cells were dotted onto nitrocellulose (Schleicher and Schuell) in the presence of 15 × SSC using a hybridot manifold apparatus (Bethesda Research Laboratories). To demonstrate that equivalent amounts of RNA were present a ^{32}P-labelled β-actin probe was used, a 2000 bp *Pst*I fragment containing the chicken β-actin cDNA (Cleveland *et al.*, 1980) which was kindly donated by C.Doni, ZMBH, Heidelberg. β-$_2$Microglobulin-specific RNA was detected with a ^{32}P-labelled β_2m cDNA, a 2000 bp *Bam*HI fragment which was a kind gift from Dr P.Kourilsky. K^k- and K^b-specific RNA was detected by a ^{32}P-labelled oligonucleotide corresponding to aminoacids 61–67 of K^k (sequence: 3′-CCC TCG CCT TGT CGG TCT AGC GG-5′) (Machy *et al.*, 1986) or to aminoacids 150–157 of K^b (sequence: 3′-CGA CCA CTT CGT CTC TCT GAG TC-5′) (Miyada *et al.*, 1985) respectively. After prehybridization and hybridization with the β-actin probe or the β_2m probe (Wahl *et al.*, 1979; Maniatis *et al.*, 1982) or with the oligo probes (Collins and Hunsaker, 1985) dot blots were exposed with Kodak XAR-film.

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