

## EFFICIENT CELL SURFACE EXPRESSION OF CLASS II MHC MOLECULES IN THE ABSENCE OF ASSOCIATED INVARIANT CHAIN

BY JIM MILLER AND RONALD N. GERMAIN

*From the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892*

Class II, or Ia, major histocompatibility complex molecules are noncovalently associated, transmembrane heterodimers consisting of two highly polymorphic glycoproteins, the 34 kD  $\alpha$  and 28 kD  $\beta$  chains, both encoded, in the mouse on chromosome 17 (1). During biosynthesis, these proteins are complexed with a nonpolymorphic polypeptide referred to as the invariant chain (Ii)<sup>1</sup> (2, 3), encoded by an unlinked gene on mouse chromosome 18 (4, 5). Several different forms of murine Ii have been described: a major 31 kD protein, a basic form (p41) (6), a series of acidic variants that result from differential sialation (7, 8), a chondroitin sulfate proteoglycan form (9), and several smaller polypeptides (p28, p20, and p10) (10). These forms of Ii are all structurally related and are probably encoded by a single Ii gene (11).

The association of Ii with Ia occurs rapidly after  $\alpha$  and  $\beta$  polypeptides are translated in the rough endoplasmic reticulum and the molecules remain complexed during cytoplasmic transport through the Golgi apparatus, where high-mannose side chains are converted to complex oligosaccharides. Ii disassociates from the Ia heterodimer just before insertion of Ia into the plasma membrane (12, 13). Although the exact fate of Ii after disassociation is unknown, some Ii has been serologically detected on the cell surface (8, 13, 14) and the proteoglycan form of Ii appears to be transiently associated with cell surface Ia (15). However, the majority of Ia on the cell surface does not appear to be associated with Ii.

This specific interaction between Ia and Ii during their biosynthesis, as well as the coordinate increase in their expression following IFN- $\gamma$  activation of a variety of cell types (16–18), has led to the hypothesis that Ii is required for the assembly, posttranslational modification, and/or cytoplasmic transport of Ia molecules (6, 8, 12, 13). Long, et al. (19) and Claesson-Welsh and Peterson (20) have shown that Ia  $\alpha\beta$  heterodimers form in the absence of Ii in *Xenopus* oocytes injected with class II mRNA, suggesting that Ii is not required for the initial association of  $\alpha$  and  $\beta$  chains. However, because all cells analyzed to date that express endogenous Ia molecules also express Ii (21), the postulated requirement for Ii in mediating cell surface expression of Ia has not been directly examined. Therefore, we have studied the expression of Ia molecules after both transient

J. Miller was supported by a Helen Hay Whitney Fellowship.

<sup>1</sup> *Abbreviations used in this paper:* GAMIg, goat anti-mouse immunoglobulin; Ii, invariant chain; SV40, simian virus 40.

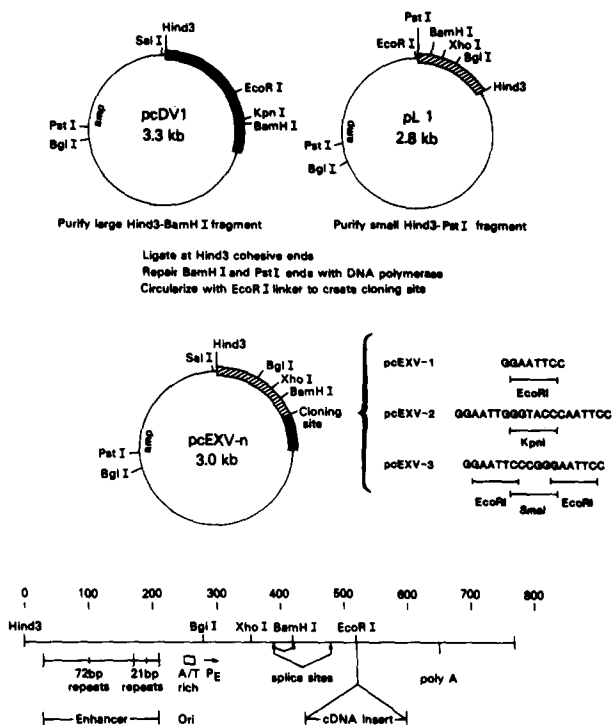


FIGURE 1. Construction of the cDNA expression vector, pcEXV-3. pcEXV-1 was derived from pcDV1 and pL1 (22) as shown, and a unique EcoRI site was generated. This cloning site was subsequently modified by the addition of synthetic oligonucleotides to generate a unique KpnI site (pcEXV-2) or a unique SmaI site flanked on either side by EcoRI sites (pcEXV-3). The linear map at the bottom of the figure is a description of the SV40 sequences (filled box in pcDV1 and hatched box in pL1) that are present in pcEXV-n. These sequences contain the SV40 enhancer, replication origin (*Ori*), early promoter (*P<sub>E</sub>*), and splice signals 5' of the cDNA insert as well as a polyadenylation signal [poly(a)] downstream. The remaining sequences in pcEXV-n are derived from pBR322 and contain the ampicillin resistance gene (*amp*).

and stable transfection of Ia cDNA clones into cells that do not express any endogenous Ii. Our demonstration that plasma membrane expression of Ia occurs efficiently in the absence of Ii indicates that the postulated obligatory role of Ii in the transport of Ia to the cell surface is incorrect.

## Materials and Methods

*Isolation of Expressible cDNA Clones for Ia and Ii.* The cDNA expression vector, pcEXV-3, was constructed from pL1 and pcD1 (22) as described in Fig. 1. This vector uses the SV40 (simian virus 40) enhancer and early promoter to transcribe inserted cDNA. It also supplies appropriate splice sites in the 5' untranslated region and a polyadenylation site 3' of the insert. Oligo (dT)-purified RNA (polyadenylated mRNA) from the Ia-expressing B cell hybridoma, LK-35.2 (H-2<sup>d</sup> × H-2<sup>k</sup>), was used to construct a cDNA library in pcEXV-3 as described (23). When this library was screened with probes for A<sub>β</sub>, E<sub>α</sub>, and E<sub>β</sub>, full-length cDNA copies of each gene were isolated. The cDNA clones representing the A<sub>β</sub><sup>d</sup> and E<sub>β</sub><sup>d</sup> alleles were distinguished from those derived from the k alleles by restriction site polymorphisms. The allelic origin of the full-length cDNA clone from the relatively nonpolymorphic E<sub>α</sub> locus could not be determined from available

nucleotide sequence information. Full-length murine Ii cDNA clones were also isolated by cross-species hybridization to the human Ii cDNA clone, p33-1 (24). Where necessary, inserts were reinserted in the correct orientation relative to the SV40 promoter to permit expression after transfection into eukaryotic cells. A full-length  $A_\alpha^d$  cDNA clone (25) was provided by Dr. Mark Davis (Stanford Univ., Stanford, CA) and inserted into pcEXV-3.

**Cell Culture.** COS-7 (26) and BALB/c 3T3 cells were maintained in DMEM (M.A. Bioproducts, Bethesda, MD) containing 10% FCS (HyClone Laboratories, Logan, UT), 2 mM glutamine, 0.1 mM nonessential amino acids, 15 mM Hepes, and 40  $\mu$ g/ml gentamycin. The cells were passed after treatment with trypsin-EDTA (Gibco, Grand Island, NY). LK-35.2 (27) cells were cultured in RPMI-1640 medium containing the above supplements plus 50  $\mu$ M 2-ME. Cell-free culture supernatants from the following mAb-producing cell lines were used for cell surface staining: M5/114 (anti- $A_\beta^d$ ) (28), 14.4.4S (anti- $E_\alpha$ ) (29), 25.9.17 (anti- $A_\beta^d A_\alpha^d$ ) (30), 39H and 82C (anti- $A_\beta^d A_\alpha^d$ ,  $A_\beta^d A_\beta^d$ ) (31).

**DNA-mediated Gene Transfer.** COS-7 cells ( $0.5 \times 10^6$  cells per 60-mm dish) were transfected in 2 ml with 2  $\mu$ g/ml plasmid DNA (1:1 ratio of  $\alpha\beta$  cDNA clones) and 500  $\mu$ g/ml DEAE-dextran, followed 3–4 h later by a 20% glycerol shock (32). The cells were harvested for analysis 48–60 h after transfection. BALB/c 3T3 cells were transfected by calcium phosphate precipitation as described for murine L cells (33), except that the DNA concentration was 10  $\mu$ g/ml of purified plasmid DNA without carrier DNA. The calcium phosphate/DNA precipitate was left on the cells overnight, the medium was replaced, and the cells were left for an additional 24 h before selection in 500  $\mu$ g/ml G418 (Geneticin; Gibco). Transfected cells were stained with the indicated mAbs followed by fluoresceinated goat anti-mouse immunoglobulin (FITC-GAM1g) (Cappel Laboratories, Cochranville, PA) and were analyzed on an EPICS V flow microfluorimeter (Coulter Electronics, Hialeah, FL) as described (34). Stable transfectants expressing surface Ia molecules were selected by preparative flow microfluorimetry.

**Northern and Southern Blots.** Cytoplasmic RNA (35) and genomic DNA (36) were prepared from tissue culture cell lines essentially as described. RNA from EBV-BLCL-45.1 was provided by Dr. Eric Long (Laboratory of Immunology, National Institute of Allergy and Infectious Diseases). Unfractionated RNA was electrophoresed on a 1% agarose-formaldehyde gel (37). Restriction enzyme-digested DNA was electrophoresed on a 0.8% agarose gel. RNA (38) and DNA (39) were transferred from the gels to nitrocellulose and the filters were hybridized to nick-translated (40) inserts from cDNA clones as indicated in the figure legends.

## Results

**Identification of  $Ii^-$  Cell Lines.** To analyze the cell surface expression of Ia in the absence of Ii, it was first necessary to identify recipient cell lines that were suitable for transfection, but did not express any endogenous Ii. Although it is unusual for an  $Ia^-$  cell to produce Ii, murine L cells, which have been used extensively for DNA-mediated gene transfer, do express the endogenous Ii gene (21). Therefore, two other  $Ia^-$  in vitro cell lines useful for transfection studies were examined for the presence of Ii mRNA: COS-7 and BALB/c 3T3.

The transformed African green monkey fibroblast line, COS-7, is especially suited for transient expression studies with the pcEXV expression system (see Materials and Methods). These cells express the SV40 large T antigen, which permits high level episomal replication of the SV40 origin-containing pcEXV plasmid. The combination of highly amplified DNA and efficient transcription from the SV40 enhancer and early promoter results in significant transient expression of proteins encoded by transfected cDNA clones (23).

Because a homologous African green monkey Ii probe was not available to assay for the expression of endogenous Ii mRNA in COS-7 cells, a human Ii probe, p33-1, (24) was tested for its ability to hybridize across this species

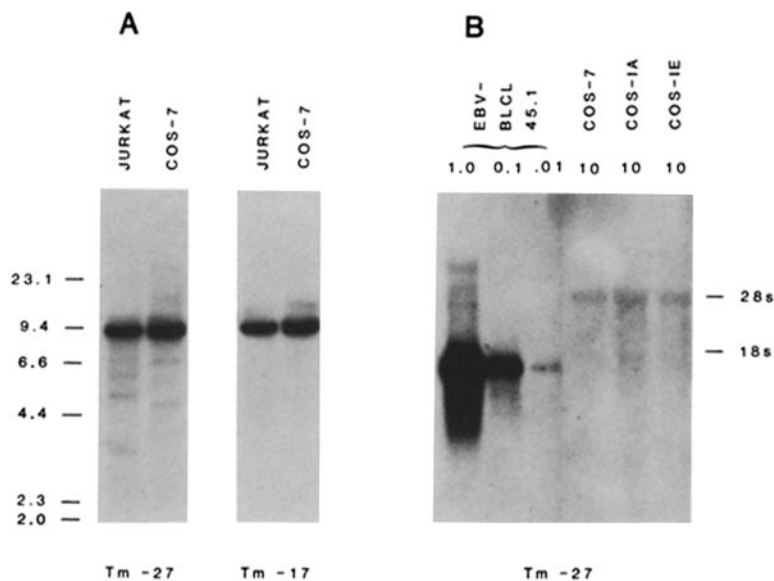


FIGURE 2. COS-7 cells do not express any endogenous Ii mRNA. (A) Southern blot containing Eco RI-digested DNA from the human cell line, Jurkat, and the monkey cell line, COS-7, hybridized to the human Ii cDNA clone, p33-1 (24). The blots were washed under low stringency (Tm-27°C) or high stringency (Tm-17°C). The sizes (in kb) of the Hind III-digested  $\lambda$  DNA marker are given on the left. (B) Northern blot containing 1.0, 0.1, and 0.01  $\mu$ g of RNA from the EBV-transformed B lymphoblastoid cell line (EBV-BLCL 45.1) and 10  $\mu$ g of RNA from COS-7 cells or COS-7 cells transfected with pcEXV-A $_{\alpha}$  plus pcEXV-A $_{\beta}$  (COS-IA) or pcEXV-E $_{\alpha}$  plus pcEXV-E $_{\beta}$  (COS-IE) cDNA clones. The blot is hybridized to the same probe as in A and washed under low stringency. The positions of 28 S and 18 S ribosomal RNA (rRNA) are shown at the right.

difference. Fig. 2A shows that the human Ii probe hybridized as efficiently to COS-7 DNA as to DNA from the human Jurkat cell line. In addition, this cross-species hybridization was not diminished when the filter was washed at high stringency (Tm-17°C) as compared to low stringency (Tm-27°C). Therefore, the human Ii probe was appropriate for examining the expression of the endogenous Ii gene in COS-7 cells.

When this same human probe was hybridized to a Northern blot, no detectable mRNA homologous to Ii was detected in 10  $\mu$ g of COS-7 RNA (Fig. 2B), even when the filter was exposed for extremely long times (not shown). However, specific hybridization to Ii mRNA was detected with as little as 0.01  $\mu$ g of RNA from an EBV-transformed human B cell line. Thus, under hybridization conditions with a human Ii probe that clearly detect cross-species hybridization to COS-7 DNA, no mRNA specific for the endogenous Ii gene was detected in COS-7 cells.

The second cell line, BALB/c 3T3, is suitable for calcium phosphate-mediated stable transfection. Unlike the Ii<sup>+</sup> L cell line, BALB/c 3T3 cells do not express any endogenous Ii. As shown in Fig. 3, when a full-length murine Ii cDNA probe was used, no mRNA for Ii was detected in 10  $\mu$ g of RNA from BALB/c 3T3 cells, whereas specific hybridization was evident with as little as 0.1  $\mu$ g of RNA from the B cell hybridoma, LK-35.2. When the filter was exposed for an extended

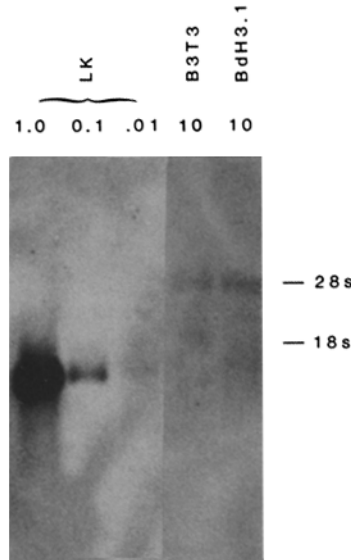


FIGURE 3. BALB/c 3T3 cells do not express any endogenous Ii mRNA. Northern blot containing 1.0, 0.1, and 0.01  $\mu\text{g}$  of RNA from LK-35.2 (LK) cells, 10  $\mu\text{g}$  of RNA from BALB/c 3T3 (B3T3) cells and 10  $\mu\text{g}$  of RNA from the transfectant, BdH3.1, was hybridized to a full-length murine Ii cDNA clone. The positions of 28 S and 18 S rRNA are shown at the right.

period of time (not shown), specific hybridization was detected with 0.01  $\mu\text{g}$  of LK-35.2 RNA, and yet no Ii mRNA was evident in BALB/c 3T3 cell RNA.

Because there is no detectable expression of the endogenous Ii gene in both COS-7 and BALB/c 3T3 cells, DNA-mediated gene transfer of Ia into either of these recipient cells could be used to test the hypothesis that Ii is necessary for the cell surface expression of Ia.

**Transient Expression Assays.** As a first test for the assembly and expression of Ia in the absence of detectable Ii, full-length cDNA clones inserted in pcEXV-3, pcEXV- $A_\beta^d$  and pcEXV- $A_\alpha^d$  ( $A_\beta A_\alpha$ , I-A) or pcEXV- $E_\beta^d$  and pcEXV- $E_\alpha$  ( $E_\beta E_\alpha$ , I-E) were cotransfected into COS-7 cells. When these cells were subsequently assayed for the presence of Ia molecules on the cell surface, significant amounts of  $A_\beta^d A_\alpha^d$  or  $E_\beta^d E_\alpha$  molecules were detected (Fig. 4). Only background staining was observed with untransfected COS-7 cells, cells transfected with vector DNA, or cells transfected with only an  $\alpha$  or  $\beta$  chain cDNA clone. In 20 separate experiments, the mean percentage of cells expressing Ia was  $11.8 \pm 7.7\%$ , with a range of 2.8–35.8%. To investigate the possibility that transfection of Ia induced the expression of the endogenous Ii gene, COS-7 cells transfected with class II  $\alpha$  and  $\beta$  cDNA clones were analyzed for the expression of Ii mRNA. As can be seen in Fig. 2B, the Ii gene was not induced in these cells. Therefore it appears that under these conditions, the surface expression of Ia requires both the  $\alpha$  and  $\beta$  chains of Ia, but not Ii.

**Stable Transfection System.** Although the above results suggest that the presence of Ii is not obligate for Ia expression, it should be noted that in the transient expression system, extremely high levels of mRNA are generated from the

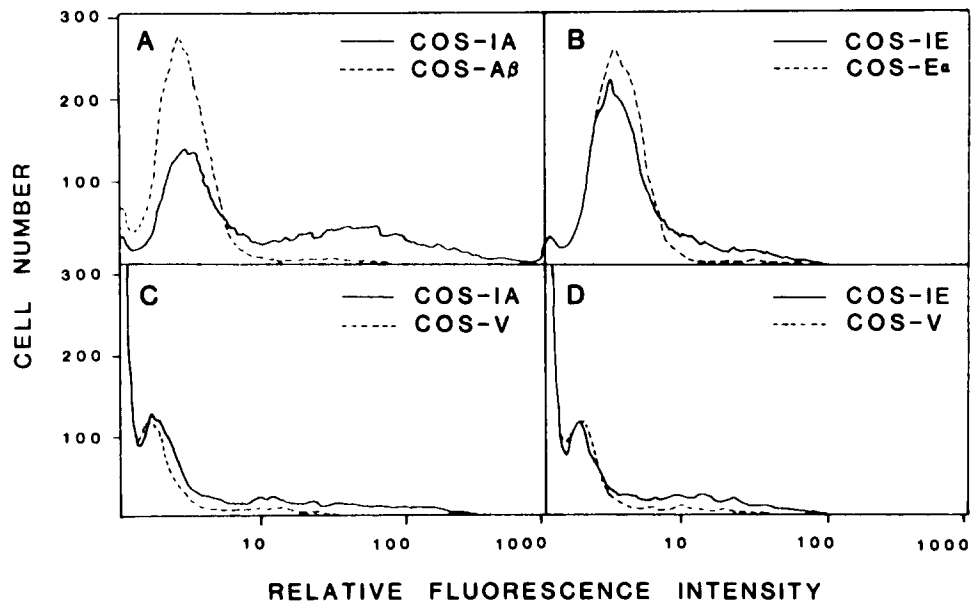


FIGURE 4. COS-7 cell transfectants express surface Ia molecules. Flow microfluorometric analysis of COS-7 cells transfected with pcEXV- $A\alpha^d$  plus pcEXV- $A\beta^d$  (COS-IA) and stained with M5/114 (solid lines in A and C) or with pcEXV- $E\alpha$  plus pcEXV- $E\beta^d$  (COS-IE) and stained with 14.4.4S (solid lines in B and D). Control transfections (dashed lines) with single  $\alpha$  (COS- $E\alpha$ ) or  $\beta$  (COS- $A\beta$ ) cDNA clones in A and B or with the pcEXV-3 vector without an insert (COS-V) in C and D were stained with the same reagents. The position of the negative peak differs between the upper panels and the lower panels because the samples were analyzed at different gains. The percentage of positive staining cells in each panel is: A, 36%; B, 11%; C, 15%; D, 11%.

transfected cDNA clones, because of the episomal amplification of input DNA and the efficient transcription from the viral promoter. Consistent with this, the small percent of transfected cells ( $\sim 10\%$ , as detected by surface Ia expression) produced a total of 5–10-fold more Ia mRNA than the B cell hybridoma, LK-35.2 (not shown). Thus, it is possible that an individual Ia-expressing COS-7 cell transfectant may contain as much as 100 times the amount of mRNA present in the LK-35.2 cells. If the role of Ii is to facilitate Ia expression, the high levels of  $\alpha$  and  $\beta$  chain precursors in such cells may account for the surface expression of Ia in the absence of Ii. Therefore, to examine the relative efficiency of Ia expression in the absence of Ii, a stable transfection system was used to limit the levels of mRNA expressed in individual cells.

To achieve this goal, pcEXV- $A\alpha^d$ , pcEXV- $A\beta^d$ , and a plasmid carrying the neomycin-resistance gene were cotransfected into BALB/c 3T3 cells. G418-resistant transfectants expressing  $A\beta^d A\alpha^d$  on the cell surface were selected by preparative flow microfluorimetry and cloned by limiting dilution. Analysis of one such clone (BdH3.1) is shown in Fig. 5. When stained with the mAb 25.9.17, which is specific for  $A\beta^d A\alpha^d$  molecules, the BdH3.1 cells were twice as bright as LK-35.2 cells, whereas equivalent staining was detected with mAb 39H or 82C, which detect both  $A\beta^d A\alpha^d$  and  $A\beta^k A\alpha^k$  molecules. This suggests that BdH3.1 ( $A\beta^d A\alpha^d$ ) and LK-35.2 ( $A\beta^d A\alpha^d$ ) and  $A\beta^k A\alpha^k$ ) express equivalent total levels of  $A\beta A\alpha$  molecules

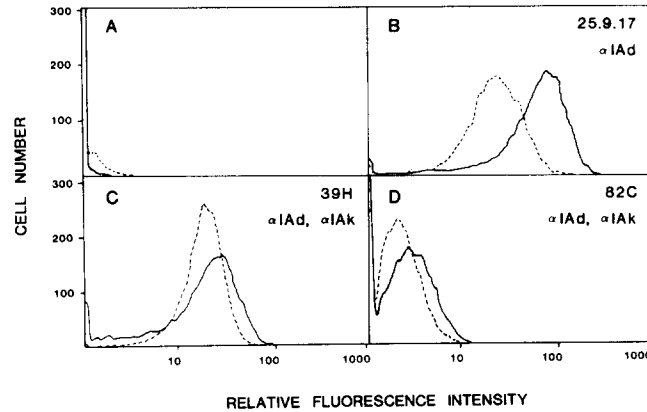


FIGURE 5. Stable expression of surface Ia molecules in BdH3.1. Flow microfluorometric analysis of LK-35.2 cells (*dashed lines*) and BdH3.1 cells (*solid lines*) after staining with (A) FITC-GAM1g only; (B-D) the indicated monoclonal antibodies followed by FITC-GAM1g.

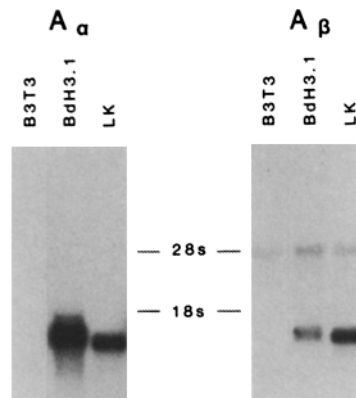


FIGURE 6. Comparable levels of Ia mRNA in LK-35.2 and BdH3.1 cells. Northern blots containing 4.0 µg RNA from BALB/c 3T3, BdH3.1, and LK-35.2 cells were hybridized to  $A_{\alpha}$  or  $A_{\beta}$  cDNA probes. Positions of 28 S and 18 S rRNA are shown.

on the cell surface. Analogous results were obtained when the cells were stained with five additional mAbs (not shown), implying that the Ia molecules on the  $Ii^{-}$  BdH3.1 cells were not only present in similar amounts, but were also structurally similar to those on the  $Ii^{+}$  LK-35.2 cells. Because  $Ii$  mRNA was not detectable in BdH3.1 cells (Fig. 3), it appears that  $Ii$  is not necessary for the stable expression of Ia molecules on the cell surface.

To examine the relative efficiency of Ia expression in these two cell types, the ratio of  $\alpha$  and  $\beta$  mRNA levels to surface Ia levels was analyzed. Northern blots hybridized to  $A_{\alpha}$ - and  $A_{\beta}$ -specific probes are shown in Fig. 6. Although BdH3.1 contains severalfold more  $A_{\alpha}$  mRNA than LK-35.2, it produces slightly less  $A_{\beta}$  mRNA. Therefore, BdH3.1 and LK-35.2 cells, which express equivalent amounts of  $A_{\beta}A_{\alpha}$  molecules on the cell surface, have comparable overall levels of  $A_{\beta}$  and  $A_{\alpha}$  mRNA, suggesting that the presence (LK-35.2) or absence (BdH3.1) of  $Ii$  does not greatly affect the efficiency of cell surface expression of class II molecules.

### Discussion

Based on previous observations, many investigators have postulated an obligate role for Ii in the biosynthesis and/or surface expression of Ia molecules (6, 8, 12, 13). The observation that Ii associates with Ia heterodimers shortly after  $\alpha$ - and  $\beta$ -chain translation and remains complexed during transport through the Golgi apparatus has been interpreted as indicating that Ii may aid in the assembly of  $\alpha$  and  $\beta$  chains or affect the specificity and/or rate of posttranslational processing of these glycoproteins. Alternatively, the finding that Ii dissociates from Ia just before membrane expression of Ia has led to the suggestion that Ii is a carrier protein that guides Ia from the endoplasmic reticulum through the Golgi and to the cell surface. Both of these hypotheses are based on correlative observations and not on direct experimental tests. Previous class II gene transfer experiments did not provide new insights into these issues, inasmuch as the recipient cells were either B lymphoma cells (41), macrophages (42), or murine L cells (43, 44), all of which express endogenous Ii (21). The experiments presented in this report specifically examine the cell surface expression of Ia after transfection of class II  $\alpha$  and  $\beta$  genes into cells that do not express any detectable endogenous Ii.

These results clearly show that significant surface expression of Ia molecules was detected in the absence of Ii after both transient expression in COS-7 cells and stable expression in BALB/c 3T3 cells. Furthermore, the similarity of the ratio of class II mRNA to cell surface Ia expression in the Ii<sup>-</sup> BALB/c 3T3 transfectants and in the Ii<sup>+</sup> B cell hybridoma indicates that the overall efficiency of Ia expression was equivalent in the presence or absence of Ii. However, this latter result refers only to steady-state levels of both mRNA and surface protein and does not exclude widely disparate Ia turnover rates in the two cell types. Thus, it is possible that the transport of Ia to the cell surface in the BdH3.1 cells might be very inefficient in the absence of Ii, but because the turnover rate of membrane-bound Ia molecules may be slow, the result would be the same amount of surface Ia on BdH3.1 as on LK-35.2 cells. Pulse-chase studies to determine the rate of Ia biosynthesis and degradation in the presence or absence of Ii are underway to address this issue.

Taken as a whole, the results of these experiments indicate that the postulated strict requirement for Ii in the formation of  $\alpha$  and  $\beta$  chain heterodimers and/or in the subsequent transport of these glycoproteins to the cell surface does not exist. These findings are consistent with recent studies examining the assembly and glycosylation of  $\alpha$  and  $\beta$  chains after injection of hybrid selected Ia mRNA into *Xenopus* oocytes (19, 20). Coinjection of Ii mRNA did not noticeably affect the assembly of  $\alpha$  and  $\beta$  chains, although it did appear to increase the rate of transport of Ia into the Golgi.

The possibility that Ii functions to control posttranslational processing of class II molecules without affecting the overall level of Ia expression has only been addressed indirectly in the transfection experiments presented here. The serologic profile generated by eight different monoclonal antibodies specific for the Ia molecules expressed by the Ii<sup>-</sup> BdH3.1 transfectant is indistinguishable from that of similarly transfected mouse L cells or of B cell hybridomas, both of which express Ii. This suggests that there are no gross structural differences between



Ia molecules synthesized in cells with or without Ii. Additional biochemical analysis will be required to determine if any differences in posttranslational modifications exist in the presence or absence of Ii.

Perhaps the most intriguing alternative for Ii function involves a role in T cell recognition of class II molecules in conjunction with antigen. Most soluble antigens require intracellular denaturation and/or degradation before recognition by T cells, and this antigen-processing pathway appears to require the low-pH environment of endosomes or lysosomes (45). Recently, it has been shown that minimal immunogenic peptides physically bind to Ia in a functionally relevant manner (46). Cresswell (47) has also shown that newly internalized receptosomes containing transferrin-neuraminidase conjugates can fuse with exocytic vesicles carrying Ia-Ii complexes to the cell surface. Taken together, these various observations raise the possibility that Ii plays a role in determining the association of antigen with Ia in this intracellular compartment. Whether the role of Ii would be to actually enhance antigen-Ia binding or to direct Ia (re)cycling through the appropriate subcellular pathway to permit interaction with processed antigen is unclear. Nevertheless, this hypothesis suggests that examination of a possible role for Ii in the functional recognition of antigen and Ia by T cells may prove fruitful.

### Summary

The intracytoplasmic forms of class II (or Ia) major histocompatibility complex heterodimers are associated with a third glycoprotein, termed the invariant chain (Ii). This specific interaction has led to the view that Ii plays a necessary role in the assembly, intracellular transport, and/or membrane insertion of Ia molecules. To test this hypothesis directly, we have transfected complementary DNA clones that encode murine class II  $\alpha$  and  $\beta$  chains into cells that do not express any endogenous Ii messenger RNA (mRNA) (COS-7 and BALB/c 3T3 cells). After DNA-mediated gene transfer, significant cell surface expression of Ia was observed in transient expression assays using COS-7 cells and a stable expression system using BALB/c 3T3 cells. Furthermore, the total levels of class II  $\alpha$  and  $\beta$  mRNA were similar in Ii<sup>-</sup> cells (transfected BALB/c 3T3) and in Ii<sup>+</sup> cells (B cell hybridoma) that expressed nearly identical amounts of surface Ia, suggesting that the efficiency of Ia expression was equivalent in the two cell types and, therefore, independent of Ii. These results indicate that the physiologic role for Ii is not simply to mediate membrane expression of Ia molecules, and that alternative hypotheses concerning the true function of this molecule need to be considered.

We thank J. Tou for technical assistance, A. Palini for flow cytometry, N. Braunstein, A. Sant, and R. Schwartz for critical comments on this manuscript, and S. Starnes for editorial assistance.

*Received for publication 23 June 1986.*

*Note added in proof:* Results similar to those described in this paper have also been obtained by Sekaly, R. P., C. Tonnelle, M. Strubin, B. Mach, and E. O. Long in studies of Ia expression after transfection of human genes (*J. Exp. Med.* 164:1490).

## References

1. Kaufman, J. C. Auffray, A. Korman, D. Schackelford, and J. Strominger. 1984. The class II molecules of the human and murine major histocompatibility complex. *Cell*. 36:1.
2. Jones, P., D. Murphy, D. Hewgill, and H. McDevitt. 1979. Detection of a common polypeptide chain in I-A and I-E subregion immunoprecipitates. *Mol. Immunol.* 16:51.
3. Long, E. 1985. In search of a function for the invariant chain associated with Ia antigens. *Surv. Immunol.* 4:27.
4. Yamamoto, K., G. Floyd-Smith, U. Francke, N. Koch, W. Lauer, B. Dobberstein, R. Schafer, and G. Hammerling. 1985. The gene encoding the Ia-associated invariant chain is located on chromosome 18 in the mouse. *Immunogenetics.* 21:83.
5. Richards, J., D. Pravtcheva, C. Day, F. Ruddle, and P. Jones. 1985. Murine invariant chain gene: chromosomal assignment and segregation in recombinant inbred strains. *Immunogenetics.* 22:193.
6. Sung, E., and P. Jones. 1981. The invariant chain of murine Ia antigens: its glycosylation, abundance, and subcellular localization. *Mol. Immunol.* 18:899.
7. Machamer, C., and P. Cresswell. 1982. Biosynthesis and glycosylation of the invariant chain associated with HLA-DR antigens. *J. Immunol.* 129:2564.
8. Claesson, L., and P. Peterson. 1983. Association of human  $\gamma$  chain with class II transplantation antigens during intracellular transport. *Biochemistry.* 22:3206.
9. Sant, A., S. Cullen, K. Giacometto, and B. Schwartz. 1985. Invariant chain in the core protein of the Ia-associated chondroitin sulfate proteoglycan. *J. Exp. Med.* 162:1916.
10. Zecher, R., W. Ballhausen, K. Reske, D. Linder, M. Schluter, and S. Stirm. 1984. The invariant chains of mouse class II antigens: biochemical properties and molecular relationship. *Eur. J. Immunol.* 14:511.
11. Yamamoto, K., N. Koch, M. Steinmetz, and G. Hammerling. 1985. One gene encodes two distinct Ia-associated invariant chains. *J. Immunol.* 134:3461.
12. Kvist, S., K. Wiman, L. Claesson, P. Peterson, and B. Dobberstein. 1982. Membrane insertion and oligomeric assembly of HLA-DR histocompatibility antigens. *Cell.* 29:61.
13. Holt, G., S. Swiedler, J. Freed, and G. Hart. 1985. Murine Ia-associated invariant chain's processing to complex oligosaccharide forms and its disassociation from the I-A<sup>k</sup> complex. *J. Immunol.* 135:399.
14. Claesson-Welsh, L., A. Scheynius, U. Tjernlund, and P. Peterson. 1986. Cell surface expression of invariant  $\gamma$ -chain of class II histocompatibility antigens in human skin. *J. Immunol.* 136:484.
15. Sant, A., S. Cullen, and B. Schwartz. 1985. Biosynthetic relationships of the chondroitin sulfate proteoglycan with Ia and invariant chain glycoproteins. *J. Immunol.* 135:416.
16. Koch, N., G. Wong, and J. Schrader. 1984. Ia antigens and associated invariant chain are induced simultaneously in lines of T-dependent mast cells by recombinant interferon- $\gamma$ . *J. Immunol.* 132:1361.
17. Collins, T., A. Korman, C. Wake, J. Boss, D. Kappes, W. Fiers, K. Ault, M. Gimbone, J. Strominger, and J. Pober. 1984. Immune interferon activates multiple class II major histocompatibility complex genes and the associated invariant chain gene in human endothelial and dermal fibroblasts. *Proc. Natl. Acad. Sci. USA.* 81:4917.
18. Momberg, F., N. Koch, P. Moller, G. Moldenhauer, G. Butcher, and G. Hammerling. 1986. Differential expression of Ia and Ia-associated invariant chain in mouse tissues after in vivo treatment with IFN- $\gamma$ . *J. Immunol.* 136:940.
19. Long, E., M. Strubin, C. Wake, N. Gross, S. Carrel, P. Goodfellow, R. Accolla, and

- B. Mach. 1983. Isolation of cDNA clones for the p33 invariant chain associated with HLA-DR antigens. *Proc. Natl. Acad. Sci. USA.* 80:5714.
20. Claesson-Welsh, L., and P. Peterson. 1985. Implications of the invariant  $\gamma$ -chain on the intracellular transport of class II histocompatibility antigens. *J. Immunol.* 135:3551.
  21. Koch, N., and A. Harris. 1984. Differential expression of the invariant chain in mouse tumor cells: relationship to B lymphoid development. *J. Immunol.* 132:12.
  22. Okyama, H., and P. Berg. 1983. A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. *Mol. Cell. Biol.* 3:280.
  23. Miller, J., T. Malek, W. Leonard, W. Greene, E. Shevach, and R. Germain. 1985. Nucleotide sequence and expression of a mouse interleukin-2 receptor cDNA. *J. Immunol.* 134:4212.
  24. Strubin, M., B. Mach, and E. Long. 1984. The complete sequence of the mRNA for the HLA-DR-associated invariant chain reveals a polypeptide with an unusual transmembrane polarity. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:869.
  25. Davis, M., D. Cohen, E. Nielson, M. Steinmetz, W. Paul, and L. Hood. 1984. Cell type specific cDNA probes and the murine I region: the localization and orientation of A<sub>d</sub>. *Proc. Natl. Acad. Sci. USA.* 81:2194.
  26. Gluzman, Y. 1981. SV40-transformed simian cells support the replication of early SV40 mutants. *Cell.* 23:175.
  27. Kappler, J., J. White, D. Wegman, E. Mustin, and P. Marrack. 1982. Antigen presentation by Ia<sup>+</sup> B cell hybridomas to H-2 restricted T cell hybridomas. *Proc. Natl. Acad. Sci. USA.* 79:3604.
  28. Bhattacharya, A., M. Dorf, and T. Springer. 1981. A shared alloantigenic determinant on Ia antigens encoded by the I-A and I-E subregions: evidence for I region gene duplication. *J. Immunol.* 127:2488.
  29. Ozato, K., N. Mayer, and D. Sachs. 1980. Hybridoma cell lines secreting monoclonal antibodies to mouse H-2 and Ia antigens. *J. Immunol.* 124:533.
  30. Ozato, K., and D. Sachs. 1981. Monoclonal antibodies to mouse MHC antigens. III. Hybridoma antibodies reacting to antigens of the H-2<sup>b</sup> haplotype reveal genetic control of isotype expression. *J. Immunol.* 126:317.
  31. Naguet, P., S. Marchetto, and P. Pierres. 1983. Dissection of the poly(GAT)-specific T-cell repertoire in H-21<sup>k</sup> mice. II. The use of monoclonal antibodies to study the recognition of Ia antigens by GAT-reactive T cell clones. *Immunogenetics.* 18:559.
  32. Lopata, M., D. Cleveland, and B. Sollner-Webb. 1984. High level transient expression of a chloramphenicol acetyl transferase gene by DEAE-dextran mediated DNA transfection coupled with dimethyl sulfoxide or glycerol shock treatment. *Nucleic Acids Res.* 12:5707.
  33. Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. Chasin. 1979. DNA-mediated transfer of the adenine phosphoribosyl transferase locus into mammalian cells. *Proc. Natl. Acad. USA.* 76:1373.
  34. Lechler, R., M. Norcross, and R. Germain. 1985. Qualitative and quantitative studies of antigen-presenting cell function using I-A-expressing L cells. *J. Immunol.* 135:2914.
  35. Mushinski, J., F. Blattner, J. Owens, F. Finkelman, S. Kessler, L. Fitzmaurice, M. Potter, and P. Tucker. 1980. Mouse immunoglobulin D: construction and characterization of a cloned delta chain cDNA. *Proc. Natl. Acad. Sci. USA.* 77:7405.
  36. Wilson, R., J. Miller, and U. Storb. 1979. Rearrangement of immunoglobulin genes. *Biochemistry.* 18:5013.
  37. Lechrach, H., D. Diamond, J. Mozney, and H. Buedtker. 1979. RNA molecular weight determination by gel electrophoresis under denaturing conditions: a critical reexamination. *Biochemistry.* 16:4743.

38. Thomas, P. 1980. Hybridization of denatured RNA and small DNA fragments transferred to intracellulose. *Proc. Natl. Acad. Sci. USA.* 77:5201.
39. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503.
40. Rigby, P., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237.
41. Germain, R., M. Norcross, and D. Margulies. 1983. Functional expression of a transfected murine class II MHC gene. *Nature (Lond.)*. 306:190.
42. Folsom, V., D. Gold, J. White, P. Marrack, J. Kappler, and S. Tonegawa. 1984. Functional and inducible expression of a transfected murine class II major histocompatibility complex gene. *Proc. Natl. Acad. Sci. USA.* 81:2045.
43. Malissen, B., M. Peele Price, J. Gorman, M. McMillon, J. White, J. Kappler, P. Marrack, A. Pierres, M. Pierres, and L. Hood. 1984. Gene transfer of H-2 class II genes: antigen presentation by mouse fibroblast and hamster B-cell lines. *Cell.* 36:319.
44. Norcross, J., D. Bentley, D. Margulies, and R. Germain. 1984. Membrane Ia expression and antigen-presenting accessory cell function of L cells transfected with class II major histocompatibility complex genes. *J. Exp. Med.* 160:1316.
45. Unanue, E. 1984. Antigen presenting function of the macrophage *Annu. Rev. Immunol.* 2:395.
46. Babbitt, R., P. Allen, G. Matseuda, E. Haber, and E. Unanue. 1985. Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature (Lond.)*. 317:359.
47. Creswell, P. 1985. Intracellular class II HLA antigens are accessible to transferring-neuraminidase conjugates internalized by receptor mediated endocytosis. *Proc. Natl. Acad. Sci. USA.* 82:8188.