CELL SURFACE EXPRESSION OF CLASS II HISTOCOMPATIBILITY ANTIGENS OCCURS IN THE ABSENCE OF THE INVARIANT CHAIN

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The class II genes of the major histocompatibility complex (MHC) encode a set of cell surface glycoproteins that are involved in several aspects of the immune response. The class II antigens, also called Ia antigens, are characterized by a high degree of allelic polymorphism and are expressed primarily on B lymphocytes, antigen-presenting cells (i.e., macrophage, dendritic cells), and human activated T lymphocytes. At least three distinct class II antigens exist in humans; these are designated HLA-DR, -DQ, and -DP (1). Each consists of two noncovalently linked subunits, the α chain (~35 kD) and the β chain (~29 kD). In addition, a third chain of ~33 kD, called the invariant (In)[1] chain, is transiently associated with the α and β subunits during their transport to the cell surface (2, 3, reviewed in reference 4). The In chain is released from the $\alpha\beta$ dimer after the three chains have been through the Golgi complex. Most of the class II antigens at the cell surface are not associated with In chain, except for a small fraction that is associated with a proteoglycan derivative of the In chain (5). The fate of the In chain, as well as its role, remains unknown. However, because it is always present in cells expressing class II antigens, and because of its physical association with class II antigens, it has been postulated, and widely assumed, that the In chain plays a role in assembly or transport of the α and β chains of class II antigens.

The isolation of mAb specific for the In chain (6, 7), as well as cDNA clones encoding the In chain (8–10), made it possible to analyze the In chain directly rather than through its association with class II antigens. Expression of the In chain in a variety of cell lines is inducible by IFN-γ, with induction kinetics similar to those of class II antigens (11), suggesting common regulatory pathways for In chain and class II gene expression, although the In chain gene is not located in the MHC (8, 12). However, Ia^- B cell variants (13, 14) and Ia^- B cells from patients with congenital immunodeficiency (15) express In chain mRNA but no mRNA from the class II genes. In these cases, therefore, a regulatory

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[1] *Abbreviations used in this paper:* DBM, diazobenzyloxymethyl; In, invariant chain; SV40, simian virus 40.

defect affects the class II genes independently of the In chain gene. In addition, certain class II–negative cells such as pre-B lymphomas, plasmacytomas, and fibroblasts express some In chain (16).

The role of In chain has already been addressed in two experimental systems without conclusive results. It was shown that α and β chains of class II antigens assemble in frog oocytes injected with mRNA for DRα and DRβ in the absence of In chain mRNA (8, 17). Unless the frog oocytes provide an analogous protein, this result ruled out an absolute requirement for In chain in assembly of $\alpha\beta$ dimers. Second, mouse L cells transfected with the murine A_α and A_β genes expressed I-A antigen at their surface (18). However, it turned out that L cells synthesize some In chain (16), making them unsuitable recipient cells for an analysis of the In chain role. In order to test whether there is an absolute requirement of the In chain for expression of class II antigens, we chose as transfection hosts cell lines that do not express any detectable endogenous In chain. These lines were transfected with cDNA clones encoding the α, β, or In chains. These cDNA clones had been constructed in an expression vector containing a simian virus 40 (SV40) promoter (19). The simian cell line COS was used to obtain transient expression, whereas stable expression of the DR antigen in absence of In chain was achieved by transfection of a human fibroblast cell line. Our results indicate that cDNAs encoding class II MHC antigens can be used successfully for cell surface expression. Most importantly, class II MHC antigen expression can occur in absence of In chain.

Materials and Methods

Cells. The human lymphoblastoid cell line 45.1 (20) was cultured in RPMI 1640 supplemented with 15% FCS (Hazelton Dutchland, Inc., Denver, PA) and 2 mM glutamine. The African green monkey kidney cell line COS and the human cell line HeLa were cultured in DMEM supplemented with 10% FCS and 2 mM glutamine. SV40-transformed human fibroblast 637B cell line (Human Genetics Mutant Depository, Camden, NJ) was cultured in Eagle's MEM supplemented with 15% FCS and 2 mM glutamine. African green monkey peripheral blood lymphocytes were obtained using a Ficoll-Paque gradient (lymphocyte separation medium; Bionetics, Kensington, MD). Briefly, 100 ml of heparinized blood was diluted with DMEM, layered on top of 15 ml of lymphocyte separation medium and centrifuged for 15 min at 800 rpm at room temperature. The interface was harvested and cells were washed twice with DMEM/10% FCS.

Antibodies. DR-FITC (Becton, Dickinson & Co., Palo Alto, CA), Leu-10-FITC (Becton, Dickinson & Co.), and B7-21 are mouse mAb that are respectively directed to determinants of DR (21), DQ (22), and DP (23). For immunoprecipitations the mAb D1-12, D4-22 (24), and BT2.9 (25) directed against monomorphic determinants of DR antigens were used.

Plasmids. All the plasmids used in the transfection experiments were isolated from a cDNA library constructed from mRNA of a human B cell line (19). The cDNA library was made in an expression vector carrying the SV40 early promoter and SV40-derived transcription termination sequences. The inserts of the clones encoding the β chains of DR, DP, and DQ antigens have been completely sequenced (19). The inserts of the clones encoding the α chains of the DR, DQ, and DP antigens, as well as the In chain, were sequenced at the 5′ end to ensure that they started upstream of the ATG translation initiation codon. The DRα insert follows a GC tail of 16 bp and contains 73 bp of 5′ untranslated sequence. The DQα insert follows a GC tail of 15 bp and contains 50 bp of 5′ untranslated sequence. The DPα insert follows a GC tail of 16 bp and contains 70 bp of 5′ untranslated sequence. The In chain insert follows a GC tail of 14 bp and starts at

the nucleotide position 9 of the mRNA sequence as determined by primer extension (26). The sequence of these cDNA clones will be described elsewhere. As a control plasmid for the transfection experiments, a cDNA clone encoding the human J chain (27) was used which had been isolated from the same cDNA library.

The DNA fragments used as probes in the hybridizations were derived from the plasmids DRα-10 (28), DRβII (29), and p33-1 (26).

Hybridizations. For Northern blots (30), RNA was denatured with glyoxal, size-fractionated in 1.5% agarose gels (31), and transferred to diazobenzyloxymethyl (DBM) paper (Schleicher & Schuell, Inc., Keene, NH). After a prehybridization in 50% formamide, 0.75 M NaCl, 25 mM sodium phosphate (pH 7.0), 5 mM EDTA, 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 1% glycine, 0.2 mg/ml sonicated and denatured salmon DNA, and 0.5% SDS for 2 h at 42°C, the filters were hybridized overnight at 42°C in the same solution but without glycine and with 10% dextran sulfate and 2×10^6 cpm/ml of denatured nick-translated probe. The final wash was in 15 mM NaCl, 1 mM sodium phosphate (pH 7.0), 1 mM EDTA, and 0.1% SDS at 65°C for 30 min unless otherwise indicated. The probes were a 868-bp Pst I fragment of the In chain cDNA clone p33-1, a 550-bp Pst I fragment of the DRα cDNA clone DRα-10, a 520-bp Pst I fragment of the DRβ cDNA clone DRβII, and a 1.8-kb Hind III–Bgl II fragment containing the first three exons of the HLA-A3 class I gene (32).

DNA-mediated Gene Transfer. All COS and 637B transfections were carried out by the calcium phosphate coprecipitation technique (33). In a typical experiment, 10^6 COS or 637B cells plated 24 h earlier in a 100-mm petri dish were transformed with 10 μg of supercoiled DNA of each α, β, In, or J chain plasmids. For stable transfections, 10 μg of the pSV2-neo plasmid was also added (34). Calcium phosphate–precipitated DNA in 1 ml was added to 5 ml DMEM/10% FCS in the petri dish and left with the cells for 16–20 h. At this time the cells were washed with DMEM and a 1-min 15% glycerol shock was applied (35). Cells were then washed three times with DMEM and incubated at 37°C in DMEM/10% FCS. For transient expression assays, cells were harvested 48 h later. For stable transfections, G-418 selection (1 mg/ml) was applied 48 h after initiation of the transfection and the medium was washed every 2–3 d.

Cell Sorter Analysis. Adherent COS or 637B cells were detached from petri dishes after incubation in Versene for 5 min at room temperature. Recovered COS cells or G-418-resistant 637B cell populations were washed in ice-cold medium containing 2% heat-inactivated serum. Transfectants were stained with either anti–class II mAb and fluoresceinated affinity chromatography–purified goat anti–mouse immunoglobulin (Becton, Dickinson & Co.) or FITC-labeled anti–class II mAb as previously described (25). Cells were washed twice with DMEM/2% FCS at 4°C and resuspended at 10^7 cells/ml in PBS/2% FCS. Fluorescence analysis and asceptic cell sorting were performed on an EPICS V (Coulter Electronics, Inc., Hialeah, FL) flow microfluorometer with log amplifier. Live cells were gated by forward light scatter as previously described (25). Data from analytical microfluorometry are displayed as graphs of cell number versus fluorescence intensity shown on a log scale. Every 85 channels represent a 10-fold increase in fluorescence arbitrary units. A minimum of 10^4 cells were analyzed per population for each determination of class II expression. For preparative cell sorting, sterile stained cells were analyzed for DR expression, and the brightest 5% cells isolated and regrown in selective medium. Homogeneous cell lines were obtained by subcloning using an auto-clone (Coulter Electronics, Inc.) single-cell deposition system.

Radiolabeling and Immunoprecipitating of the DR Molecules. Transfected COS cells and the 45.1 B cell line were labeled for 6 h at a density of 1.5×10^6 or 5×10^6 cells/ml, respectively, in DMEM lacking methionine and supplemented with 0.5% FCS, 2 mM glutamine, and [^{35}S]methionine (1,000–1,400 Ci/mmol; Amersham Corp., Arlington Heights, IL) at 250 μCi/ml. After labeling, cell pellets were solubilized in buffer containing 10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.0015 M $MgCl_2$, 0.5% NP-40, and the protease inhibitors PMSF (200 μg/ml; Sigma Chemical Co., St. Louis, MO) and 0.1% aprotinin (Sigma Chemical Co.). Lysates were spun for 1 h at 60,000 *g* in a SW50Ti rotor (Beckman Instruments, Inc., Palo Alto, CA). The supernatant of the lysate was adjusted to pH 8.0

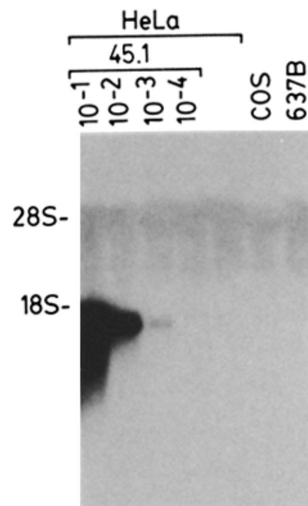

FIGURE 1. Lack of In chain mRNA in different cell lines. Total RNA from the indicated cell lines was size-fractionated by gel electrophoresis and transferred to DBM-paper. RNA from the B cell line 45.1 was serially diluted into HeLa cell RNA in 10-fold increments. 20 μg of RNA was loaded in each lane. Hybridization was with a nick-translated fragment from the In chain cDNA clone p33-1. The final wash was in 37.5 mM NaCl, 5 mM sodium phosphate (pH 7.0), 1 mM EDTA, and 0.1% SDS at 65°C for 30 min. At this stringency the p33-1 probe hybridizes weakly with 28 S rRNA. This hybridization was also observed with poly(A)$^-$ RNA and was reduced by washes at higher stringencies. The position of 18 S and 28 S RNA is indicated on the left.

with 6 μl of 1 M NaOH and to 1% aprotinin. Samples were incubated at 4°C for 4 h with 8 μl of anti-SV40 antisera (a gift of H. Turler) and 8 μl of BALB/c serum, followed by a 2-h incubation with protein A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). Samples were spun down and supernatants were incubated overnight at 4°C with 24 μl of ascitic fluids from a pool of anti-DR mAb. Samples were spun for 3 min in the Eppendorf microfuge (Brinkman Instruments, Westbury, NY) and the pellets were discarded. Protein A-Sepharose was added to the supernatant and incubation was continued for 4 h. Immunoprecipitates were washed twice in 50 mM sodium phosphate (pH 8.3), 5 mM EDTA, 0.15 M NaCl, 1% NP-40, 10 mM methionine, and 1% aprotinin, three times in the same buffer without aprotinin and with 0.5 M NaCl and twice in 50 mM sodium phosphate (pH 8.3), 1 mM EDTA, 0.15 M NaCl, and 0.5% NP-40. Two-dimensional gel electrophoresis, with nonequilibrium pH gradient electrophoresis in the first dimension, was run according to O'Farrell et al. (36). The second dimension was in 12% polyacrylamide gels. Gels were fixed in 10% TCA, treated with Enhance (New England Nuclear, Boston, MA) washed in 20% methanol and 3% glycerol, and dried. Dried gels were exposed to Kodak XS-5 film, with intensifying screens at −70°C.

Results and Discussion

The role of the In chain was investigated by transfecting the genes for the α, β, and In chains of human class II MHC antigens into cell lines that are not expressing In chain. Several cell lines known to be efficient recipients for DNA were first tested for the presence of endogenous In chain RNA. The human cervical carcinoma line HeLa and the human fibroblast line 637B were tested, as well as the African green monkey kidney line COS (Fig. 1). To define the detection limit of the experiment, RNA from the EBV-transformed B lympho-

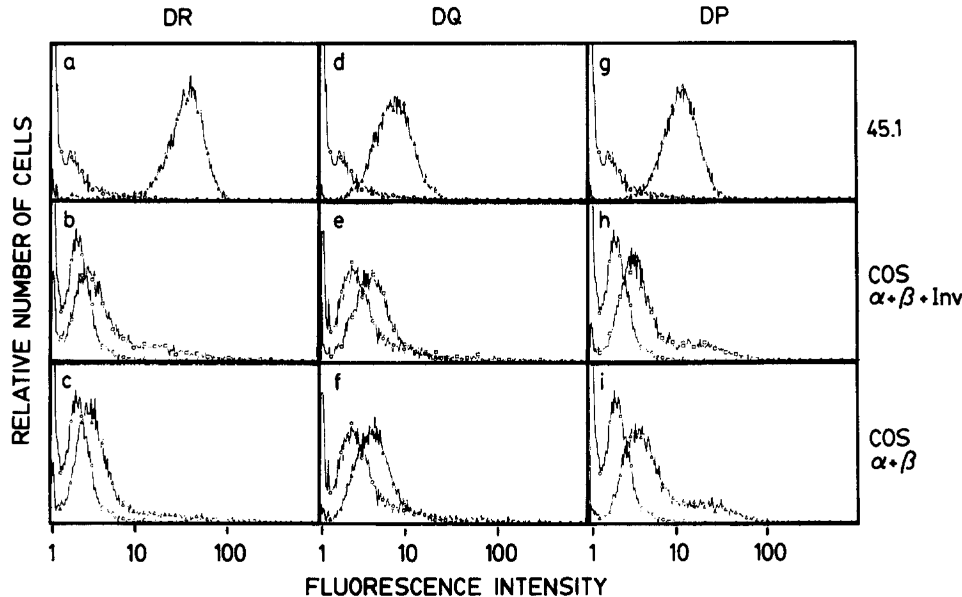

FIGURE 2. Cell surface expression of MHC class II genes cotransfected with or without In chain in COS cells. 10 μg of each plasmid were transfected in COS cells using the $CaPO_4$ precipitation technique. To keep the amount of DNA constant, 10 μg of a plasmid with a cDNA encoding the human J chain (27) was cotransfected whenever the In chain was absent. Cells were harvested 72 h after transfection, stained by direct immunofluorescence with a mAb to a monomorphic determinant of DR, and analyzed on a flow microfluorometer. The same transfection procedure was repeated in COS cells with plasmids carrying the DQα and DQβ cDNA (*e–f*) or the DPα and DPβ cDNAs (*h–i*). In each case these plasmids were transfected with (*e* and *h*) or without (*f–i*) In chain cDNA. The cell surface expression of MHC class II molcules on the surface of transfected cells was compared to that of a B cell line 45.1 (*a*, *d*, and *g*). Each panel represents a comparison between the indicated cells stained with the relevant mAb (*open triangle*), (*open square*) or with an irrelevant mAb (*open circle*). 10,000 viable cells were accumulated for each histogram.

blastoid cell line 45.1, which expresses In chain RNA, was serially diluted into HeLa RNA and included in the same gel. The results show that, if any In chain RNA is present in HeLa, 637B, or COS cells, it would have to be $<10^{-4}$ the level found in B cells. The stringency of the hybridization was chosen to allow detection of the simian In chain RNA with the human probe (see below). We concluded that the three lines, HeLa, 637B, and COS were suitable recipients for our experiments in that they express insignificant amounts of In chain RNA, if any.

Cell Surface Expression of Class II MHC Antigens in Transfected COS Cells. COS cells carry integrated copies of the SV40 T antigen gene (37). Plasmids carrying the SV40 origin of replication replicate in COS cells. We chose to test the COS system for expression of class II MHC antigen cDNA clones that had been constructed in an SV40-derived vector (19). cDNA clones encoding the DRα, DRβ, and In chains were introduced into COS cells by the calcium phosphate precipitation technique (33). 3 d after transfection, cells were analyzed for the presence of surface DR antigen by flow cytometry using a mAb to a monomorphic determinant of DR. The histograms (Fig. 2*b*) show a bimodal distribution with a majority of cells (≥70%) expressing low but significant amounts of DR antigen,

and a smaller proportion of cells (25.7%) expressing levels of DR antigen comparable to those observed in a B cell line (Fig. 2*a*). We conclude that COS cells have all the necessary components for the assembly and transport of class II antigens, and that they provide a useful and rapid assay for cell surface expression of class II antigens. Considering that no selection is applied, it is remarkable that as many as 20% of cells show bright fluorescence. Other studies in COS cells with a single gene product expressed at the cell surface reported 10–20% of positive cells (38, 39). The requirement of In chain for cell surface expression of human MHC class II molecules was investigated. When COS cells were transfected only with DRα and DRβ cDNA clones, they expressed DR antigens at the cell surface with a similar bimodal distribution (Fig. 2*c*). The results of several experiments (Table I) indicated that any difference in the mean fluorescence intensity or in the percentage of brightly fluorescent cells between cells transfected with or without the In chain was within experimental variability. Therefore, unless endogenous In chain synthesis is induced in transfected COS cells, assembly and transport to the cell surface of DR antigen do not require In chain.

In order to test whether this lack of In chain requirement also held for other class II antigens, cDNAs encoding the α and β chains of the DQ and DP antigens were introduced into COS cells with or without the In chain. Transfectants were analyzed by flow cytometry using antibodies specific for DQ and DP. The results (Fig. 2 and Table I) show that cell surface expression of DQ and DP antigens in COS cells can occur in absence of In chain of human origin.

The In Chain Is Not Induced in Transfected COS Cells. RNA from transfected COS cells was analyzed to test for the possibility of induction of the endogenous In chain. RNA isolated from peripheral blood lymphocytes of African green monkey was included as a positive control to show that the human DNA probes for the α, β, and In chains did crosshybridize to their simian counterparts. These RNA hybridizations also allowed determination of the size and abundance of the transcripts derived from the cDNA expression vectors. These transcripts are expected to be about 300 nucleotides longer than mRNAs transcribed in cells naturally expressing class II antigens because they contain about 60 nucleotides of the SV40 early leader, 45 nucleotides of a synthetic polylinker, and a G tail that varies between 6 and 18 nucleotides upstream of the cDNA insert and about 185 nucleotides of SV40 sequences downstream of the poly(A) tail (19, 40). mRNAs for the DRα, DRβ, and In chains are all ~1.3 kb (13). Therefore, transcripts from the SV40-derived expression vectors should be ~1.6 kb. Fig. 3*A* shows the hybridization with the DRα probe. The mRNA for the simian DRα chain can be detected in the lymphocytes of African green monkey and is of the same size as DRα mRNA from a human B cell line. As expected, untransfected COS cells are negative. COS cells transfected with the DRα cDNA clone express high levels of DRα transcripts, most of which are ~1.6 kb. Discrete bands at ~3.5 kb and 4 kb are also detected. These longer transcripts could result from transcription initiation or termination in the plasmid sequence, or from recombination events between vectors. Hybridizations with the DRβ and the In chain probes show similar results (Fig. 3*B* and *C*). The corresponding mRNAs in the monkey cells are easily detected, and transfected COS cells show

TABLE I

Cell Surface Expression of Class II Antigens in COS Cells

Exp.*	Plasmids‡	Mean fluorescence§	Percent of cells above channel 60
1	DRα, DRβ, In	56.8	31.6
		52.0	27.0
		40.8	16.2
1	DRα, DRβ, J	51.0	25.7
		49.9	28.2
		39.4	20.5
1	In	16.7	1.5
2	DRα, DRβ, In	48.7	21.5
		47.5	19.6
2	DRα, DRβ	47.0	20.5
		45.8	22.3
2	In	10.0	2.0
3	DRα, DRβ, In	49.8	23.0
		47.1	24.3
		41.6	20.3
3	DRα, DRβ, J	44.6	20.8
		47.4	26.4
		46.2	22.5
3	DPα, DPβ, In	57.1	40.1
3	DPα, DPβ, J	59.3	37.6
3	In	15.1	1.0
4	DPα, DPβ, In	51.7	27.3
4	DPα, DPβ	49.6	21.3
		50.4	24.5

10^6 COS cells were transfected with the indicated plasmids in duplicate or triplicate petri dishes. Cells were harvested 72 h later and stained with mAb specific to DR or DP as described in Fig. 2. FACS analysis was performed as detailed in Materials and Methods.

* To maintain the total amount of transfected DNA constant, an irrelevant plasmid was added in the transfections that did not include the In chain (experiments 1 and 3). To rule out a possible artefact caused by the irrelevant plasmid, transfections were also carried out with only the α and β chain plasmids (experiments 2 and 4).

‡ 10 μg of each plasmid was used, except when the In chain plasmid was transfected alone in which case 30 μg was used. The J chain plasmid was isolated from the same cDNA library and is described in Materials and Methods.

§ The mean fluorescence is expressed in channel numbers (arbitrary units). A twofold increase in fluorescence represents a shift of 25 channels.

primarily 1.6-kb transcripts with some 3.5- and 4-kb transcripts. Most importantly, COS cells transfected only with the DRα and DRβ vectors show no detectable In chain RNA. From these results we may conclude that the In chain is not necessary for cell surface expression of DR antigens.

The levels of DRα, DRβ, and In chain transcripts in transfected COS cells are comparable to those found in B cells (Fig. 3). In contrast, the average level of cell surface DR antigens is much lower in transfected COS cells than in B cells (Fig. 2). Several reasons could account for the apparent inefficiency of cell surface DR antigen expression in COS cells. First, the recombinant RNA tran-

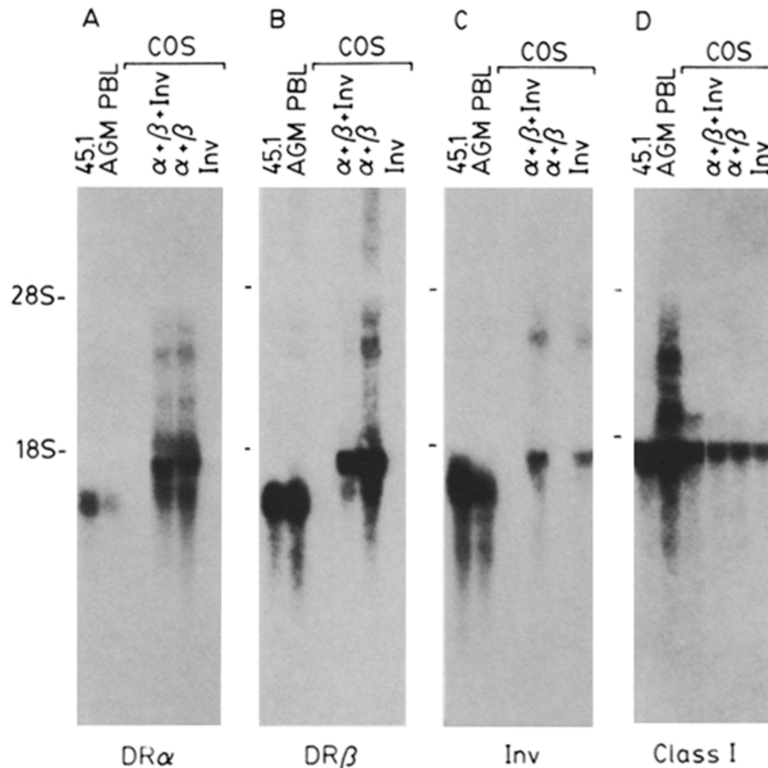

FIGURE 3. Northern blot analysis of transfected and untransfected cells. 20 μg of total RNA from the indicated cells was size-fractionated by gel electrophoresis and transferred to DBM paper. *45.1* is a human EBV-transformed B cell line; *AGM PBL* are peripheral blood lymphocytes from African green monkey; *COS* are COS cells either untransfected or transfected with plasmids carrying cDNA for DRα (α), DRβ (β), and In chain (*Inv*) in the combinations indicated. The filters were hybridized with nick-translated DNA fragments from DRα cDNA (*A*), DRβ cDNA (*B*), In chain cDNA (*C*), and an HLA-A3 gene (*D*). Hybridization with the class I probe (*D*) served to show that the RNA from untransfected COS cells was intact. The final wash was in 37.5 mM NaCl, 5 mM sodium phosphate (pH 7.0), 1 mM EDTA, and 0.1% SDS at 65°C for 30 min. The position of 18 S and 28 S RNA is indicated at the left of each panel.

scripts may be translated inefficiently because of the artificial 5′ and 3′ untranslated sequences. Second, transfected COS cells may be inherently inefficient in the posttranscriptional steps required. Finally, translation of the In chain may not occur in cells transfected with the In chain cDNA. Therefore, experiments were carried out to test for translation of the In chain in transfected COS cells.

Expression of In Chain Protein from Its cDNA in COS Cells. It was important to test whether the In chain cDNA was expressed and whether its product would associate with intracellular αβ dimers. For this purpose, immunoprecipitation of the DR molecules in cells biosynthetically labeled with [^{35}S]methionine was carried out using an mAb specific for DR antigens (Fig. 4). DR antigens immunoprecipitated from a control human B cell line show a characteristic pattern of spots on a two-dimensional gel. The DRα and DRβ chains appear as multiple spots, owing to processing of the glycosyl groups. The In chain, which is precipitated by virtue of its association with DR molecules, appears as a

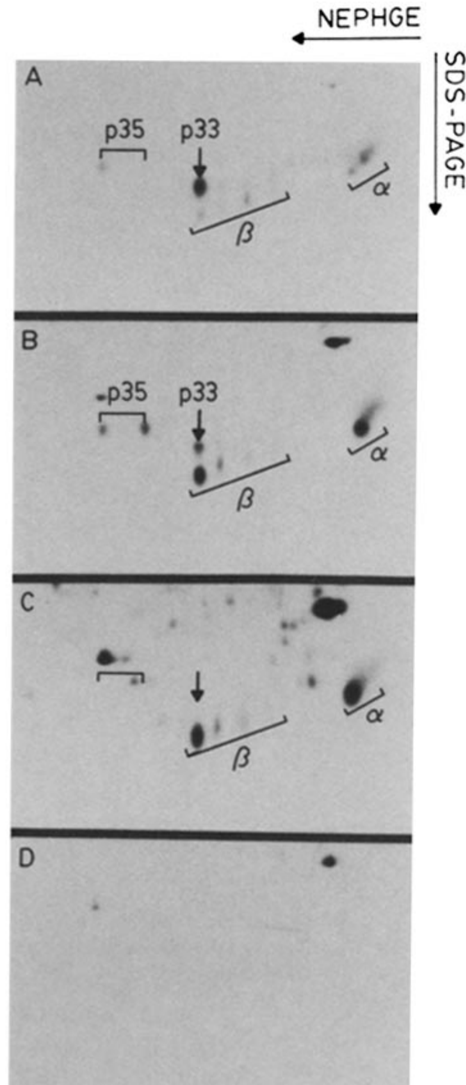

FIGURE 4. Two-dimensional gel analysis of HLA-DR antigens immunoprecipitated from a B cell line and from transfected COS cells. Cells were labeled with [^{35}S]methionine for 6 h. DR antigens were immunoprecipitated with a pool of mAbs, D1-12, D4-22, and BT2.9. The *arrow* indicates the position of the p33 In chain. *Brackets* indicate the position of α chain spots, β chain spots, and the basic p35 spots that are related to the p33 In chain. To facilitate comparisons the *arrows* and *brackets* have been placed at identical positions in the different panels. (*A*) B cell line 45.1; (*B*) COS cells transfected with DRα, DRβ, and In chain cDNA clones; (*C*) COS cells transfected with DRα and DRβ chain cDNA clones; (*D*) COS cells transfected with the In chain cDNA clone.

prominent spot of 33 kD (p33), with a row of more acidic spots that extends to the DRα chain position, and with two additional, more basic spots of 35 kD (p35). In COS cells transfected with cDNAs for the DRα, DRβ, and In chains, a similar pattern of spots is observed. Therefore translation of these three chains occurs with fidelity in COS cells even though the relative intensity of the different

spots is not identical. It is also evident that the amount of In chain associated with DR $\alpha\beta$ complexes in COS cells is lower than that in B cells. This is not a surprising result because B cells produce much more In chain than they do DRα and DRβ chains (41), whereas transfected COS cells produce equivalent levels of RNA for each chain. We cannot predict how DR expression in COS cells would be affected if much higher levels of In chain could be produced.

Immunoprecipitations from COS cells transfected with only DRα and DRβ chains show the typical α and β spots, and no material at the position of the In chain spots. Assembly and processing of the $\alpha\beta$ dimers therefore proceed in absence of the In chain. Processing of $\alpha\beta$ dimers in presence or absence of the In chain was investigated in another study in which mRNAs for the DRα, DRβ, and In chains were injected in frog oocytes (17). Although the α and β chains assembled in the absence of the In chain, it appeared that their transport was slowed and that their glycosylation patterns were incomplete. Our experiments do not address directly the question of the rate of transport and the fine specificity of posttranslation modifications. Although these parameters could be affected by the In chain, it is clear from our data that the In chain is not an absolute requirement for cell surface expression of DR antigen.

Transfected Human Fibroblasts Express DR Antigens in the Absence of the In Chain. The expression of transfected genes in COS cells is transient. Furthermore, translation efficiency of class II antigens is much lower in transfected COS cells than in B cells. To test whether the In chain was necessary for cell surface expression of class II antigens in a stable expression system, we analyzed the human fibroblast line 637B, which had been transfected with the cDNA clones for the DRα, DRβ, and In chains together with the plasmid pSV2-neo (34), which allows selection with the neomycin analog G-418. G-418-resistant colonies were pooled 3 wk after selection and were stained with a mAb specific for DR antigens. Preparative cell sorting was used to isolate a homogeneously positive population of cells. The 637B clone C9 and its subclones C9B5 and C9E8 express levels of DR antigen which are 3–10-fold lower than those found on a B cell line (Fig. 5).

Because the frequency of cells positive for DR antigens was very low among the G-418-resistant 637B cells, we reasoned that the efficiency of cotransfection with unlinked genes might be low. As a result, it was likely that a gene that was not under selection would not be expressed in the transfected cells. In order to test this possibility, we analyzed RNA from the cloned transfectants for the presence of In chain transcripts. The results (Fig. 6) showed that the 637B-C9 clone, as well as its subclones, does not express detectable In chain RNA. Therefore, the cDNA clone for the In chain was either not retained or not expressed in the transfected cells. This experiment also shows that the endogenous In chain gene is not induced in 637B cells as a result of transfection with class II genes. It was an important control because the In chain gene in 637B cells is inducible by treatment with IFN-γ (Fig. 6). Hybridizations with DRα and DRβ probes (data not shown) showed the expected 1.6-kb transcripts in the transfected 637B-C9 cells. However, the levels of DRα and DRβ transcripts were much lower than in COS cells. Unlike COS cells, transfected 637B cells lacking In chain are therefore quite efficient in the posttranscriptional steps of DR antigen expression. The results obtained with 637B cells confirm and strengthen

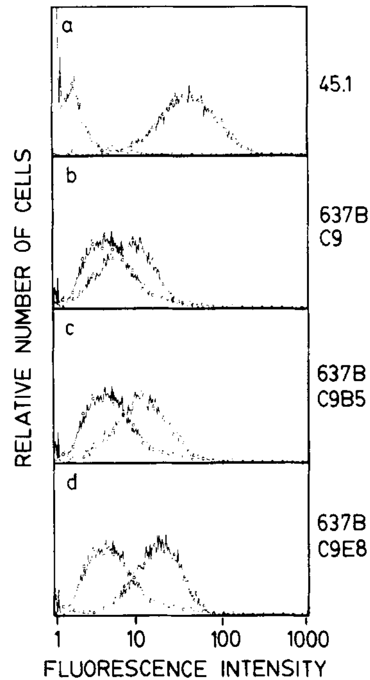

FIGURE 5. Cell surface expression of DR antigens in a human fibroblast line transfected with cDNA clones. Cloned stable transfectants of the fibroblast line 637B expressing DR antigens were obtained as described in Materials and Methods. Three of these clones (panels *b–d*) were stained with a mAb to DR and compared with a B cell line (panel *a*). Each panel represents a comparison between the indicated cells stained with the relevant mAb (*open triangle*) or with an irrelevant mAb (*open circle*). 10,000 viable cells were accumulated for each FACS histogram.

the conclusion derived from the COS cell transfections because they show that constitutive and efficient cell surface expression of DR antigens can occur in absence of the In chain.

Conclusions. This report demonstrates that class II MHC antigens can be expressed at the surface of simian and human cells in absence of the In chain. The In chain, which is associated with class II antigens in the cytoplasm of natural class II–positive cells, is therefore not required for assembly and transport of class II α and β chains. Unless the association of the In chain with class II $\alpha\beta$ dimers is fortuitous, this conclusion suggests an interesting alternative, namely, that the In chain is involved in the function of class II MHC antigens. Presentation of foreign antigens to class II–restricted T lymphocytes requires processing of antigen and most likely a direct interaction between specific peptides and class II antigens (42, 43). The nature of this interaction and the mechanisms by which it occurs are unknown, but important steps probably take place inside the cell. Recycling of class II antigens into the cell may be necessary for antigen processing and presentation. It is easy to imagine a role for the In chain in these intracellular events. Cresswell (44) has shown that class II antigens on their way to the cell surface intersect the pathway taken by endocytosed transferrin receptors before dissociation of the In chain. Therefore, class II $\alpha\beta$In complexes could interact with internalized foreign antigens in antigen-presenting cells.

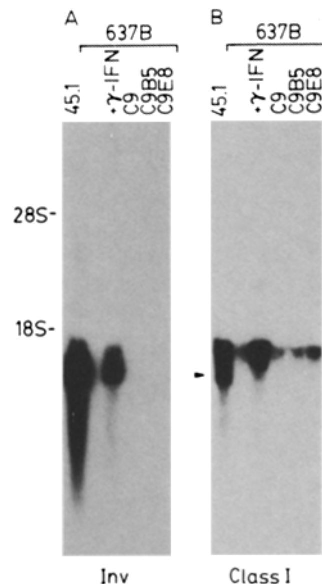

FIGURE 6. The DR$^+$ transfected cell 637B does not express the In chain gene. 20 μg of total RNA from the indicated cells was size-fractionated by gel electrophoresis and transferred to DBM paper. *45.1* is a human EBV-transformed B cell line. *637B* is a human SV40-transformed fibroblast that was grown in absence or in presence (+ *γ-IFN*) of rIFN-γ (a gift of N. Sarvar, Meloy Laboratories, Springfield, VA). *C9* is a clone of 637B cells transfected with HLA-DR genes. *C9B5* and *G9E8* are subclones of C9. (*A*) The filter was hybridized with a nick-translated DNA fragment from the In chain cDNA. (*B*) The filter shown in *A* was rehybridized with a nick-translated DNA fragment from the HLA-A3 gene. The *arrowhead* points to the residual signal from the In chain probe. Hybridization with the class I probe served to show that the RNA from all cell lines was intact. The positions of 18 S and 28 S RNA are indicated on the left.

The major conclusion from our study is that, rather unexpectedly, association with In chain is not a requirement for cell surface expression of class II $\alpha\beta$ heterodimers. The availability of transfected cells expressing class II antigens in absence of In chain and of an expressible In chain cDNA makes it possible to test what role the In chain might play.

Summary

The invariant chain is a glycoprotein transiently associated with the α and β subunits of class II antigens of the major histocompatibility complex during their transport to the cell surface. An expression assay with cDNA clones transfected into simian COS cells was used to test whether the invariant chain is required for assembly and transport of human class II antigens. COS cells do not express detectable levels of RNA from the endogenous invariant chain gene. Cell surface expression of the DP, DQ, and DR antigens was observed in COS cells transfected with the respective α and β chain cDNA clones. Analysis of RNA from the transfected cells showed that the human genes were transcribed in COS cells and that the endogenous simian class II and invariant chain genes were not induced. Cotransfections with an invariant chain cDNA clone did not alter the levels of class II antigens at the cell surface. Biosynthetic labeling and immunoprecipita-

tion demonstrated that the invariant chain cDNA was expressed into a protein which associated with DR α and β chains. Efficient expression of DR antigen in absence of invariant chain was also observed at the surface of a human fibroblast line stably transfected with DR α and β cDNA. This study demonstrates that expression of all three human class II antigens can be achieved with cDNAs cloned in expression vectors. Furthermore, cell surface expression of class II major histocompatibility complex antigens can occur in absence of invariant chain. The postulated role of the invariant chain in class II antigen transport to the cell surface must be reevaluated. The invariant chain may rather be involved in functional properties of class II molecules such as antigen presentation.

We thank A. Pallini and M. Waxdal for expert operation of the flow microfluorometer, W. Pugh for his excellent technical assistance, D. Murphy for the gift of African green monkey blood, E. Cowan for the HLA-A3 gene fragment, and H. Turler for the anti-SV40 antiserum. We are grateful to G. Shaw for her kind editorial assistance.

Received for publication 23 June 1986.

Note added in proof: Results similar to those described here have been obtained by Miller and Germain (45) with transfections of murine Ia antigen genes.

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