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Evidence for a novel HLA antigen found on human extravillous trophoblast and a choriocarcinoma cell line

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SUMMARY

We describe the characterization of a novel HLA Class I molecule, which we have isolated from chorionic cytotrophoblast cell membranes, and from a trophoblast-derived choriocarcinoma cell line, BeWo; classical HLA Class I antigens are not expressed on these cells. This antigen is an electrophoretically non-polymorphic glycoprotein of approximately 40,000 molecular weight, which is found in association with β_2 microglobulin, and which is detected by monoclonal antibodies recognizing monomorphic determinants of HLA Class I. Elucidation of the nature and origin of this molecule may provide valuable information regarding the immune barrier that exists between mother and fetus.

INTRODUCTION

The genetic complexity of the Class I MHC loci on human chromosome 6 suggests that there may be functional Class I loci in addition of HLA-A, B and C (Ploegh, Orr & Strominger, 1981), analogous to the Qa antigens in the mouse (Hood, Steinmetz & Goodenow, 1982). In the rat, a unique Class I placental antigen (Pa) has been identified on basal trophoblast that only carries broadly shared Class I antigenic determinants; expression of classical Class I antigens with private specificities is suppressed in this tissue (Macpherson *et al.*, 1986). The presence of such molecules on human trophoblast has until now not been positively demonstrated, although there is evidence to suggest that activated T cells, and T-ALL cells, may express an additional A-locus related antigen together with normal HLA-A, B and C (Orgad *et al.*, 1985).

It has been shown previously, by staining of tissue sections, that human extravillous trophoblast expresses an antigen which is detected by antibodies recognizing monomorphic determinants of HLA Class I, but not by antibodies to polymorphic determinants (Redman *et al.*, 1984). We decided to investigate this molecule further, isolated from chorionic trophoblast cells and human choriocarcinoma cell lines. Previous work has shown that the level of expression of Class I molecules varies between individual lines (Kawata *et al.*, 1984). The cell line BeWo was chosen for further investigation as our preliminary studies showed that it expresses measurable quantities of β_2m and an associated MHC heavy chain on its surface, confirming earlier reports (Trowsdale *et al.*, 1980; Anderson & Berkowitz, 1985).

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Our results showed that BeWo and extravillous chorionic cytotrophoblast express high levels of a 40,000 molecular weight (MW) β_2m-associated molecule, which was recognized by monoclonal antibodies to monomorphic determinants of HLA Class I. We have used flow cytometry, SDS–polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing (IEF) to confirm that this is a non-classical MHC Class I antigen.

MATERIALS AND METHODS

Preparation of cells for analysis

The amniochorion proved to be the best source of material for the isolation of extravillous trophoblast. The cells were prepared by enzymatic digestions of the chorion membranes after the amniotic epithelium had been removed. Decidual tissue was scraped off with a razor blade and 10-cm square pieces of membrane were incubated in 20 ml protease type 14 (Sigma, Poole, Dorset) at 1 mg/ml in RPMI-1640 with 25 mM HEPES (Flow, Irvine, Ayrshire) for 1 hr at 37° in 5% CO_2 in air. The membranes were then washed once in PBS and incubated for a further 90 min in 20 ml of solution containing 1 mg/ml collagenase type 4 and 2 mg/ml hyaluronidase type 1-S (Sigma) in Eagle's modified MEM with 25 mM HEPES (Flow) with 5% fetal calf serum (FCS). The digest was filtered through 100 μm gauze to remove undigested tissue and cell aggregates, then washed twice in PBS. Fifty donor placentas were used in this study. In each case serial cryostat sections of the amniochorion were made, followed by immunoperoxidase labelling using a panel of monoclonal antibodies. Cytospin samples were prepared from the isolated cells, and the same panel of antibodies was used to study surface antigen expression. Lymphocytes were also subjected to the same series of enzyme digestions as the

trophoblasts to show that surface HLA was not removed or altered. The HLA-A, B and C types of donors and fetuses were determined by the standard NIH technique, using mononuclear cells prepared from maternal peripheral blood and placental blood obtained directly from the umbilical vein.

Monoclonal antibodies

The percentage of trophoblast cells present in each sample was determined by labelling of cytospin preparations with the monoclonal antibody JMB2 (McGee *et al.*, 1982), which binds to intermediate filaments and for this tissue is trophoblast-specific. W6/32 (Barnstable *et al.*, 1978) binds to a monomorphic determinant of HLA Class I, and was used to demonstrate the presence of Class I by immunoperoxidase staining, by complement-mediated killing, and by immunoprecipitation. Monoclonal antibodies used to polymorphic determinants of HLA Class I were MEI (Ellis, Taylor & McMichael, 1982), MHM.5 (Ellis *et al.*, 1985), and MA2.1 (McMichael *et al.*, 1980), and BBM.1 (Brodsky, Bodmer & Parham, 1979) was used to detect β_2m. NDOG2 (Stirrat, Sunderland & Redman, 1983) was used to demonstrate the presence of placental alkaline phosphatase. NA1/34 (McMichael *et al.*, 1979), an anti-CD1, and SG171 (Goyert, Shively & Silver, 1982), an anti-HLA DR, were used as negative controls in immunoprecipitation experiments. Details of all monoclonal antibodies used are listed in Table 1.

Cell lines

The choriocarcinoma cell line BeWo (Trowsdale *et al.*, 1980) (gift of P. Goodfellow, ICRF) was maintained in DMEM medium containing 10% fetal calf serum (FCS, Gibco, Paisley, Renfrewshire). The cells were adherent, and were therefore dispersed by vigorous shaking or pipetting, or by treatment with 0·1% trypsin (Sigma). EBV-transformed B-cell lines (BCL) of known HLA type were used as controls, and were grown in RPMI-1640 (Gibco) with 10% FCS.

Analysis by flow cytometry

Cells were incubated with monoclonal antibody at 2 μg/ml for 1 hr at 4°, then washed twice with phosphate-buffered saline, pH 7·4 (PBS) plus 0·1% bovine serum albumin (BSA), 0·02% sodium azide. Fifty μl of fluorescein isothiocyanate-conjugated goat anti-mouse IgG (FITC GAM, Sigma) were then added to each sample, with the addition of 20% human AB serum to block non-specific binding, and incubation was continued for a further hour. Cells were washed twice, and analysed immediately on an Ortho Cytofluorograf (by Dr N. Carter, Nuffield Dept. of Surgery, John Radcliffe Hospital, Oxford).

Labelling of cell surface proteins with ^{125}I

The trophoblasts survive for only a few hours following preparation, and therefore the surface proteins were labelled immediately with ^{125}I using a lactoperoxidase/glucose oxidase catalysed radioiodination as described by Knowles & Bodmer (1982). Between 20×10^6 and 50×10^6 cells were labelled with 1 mCi ^{125}I, and then only if viability exceeded 85%. The cells were washed three times with PBS prior to labelling.

BeWo cells were removed from tissue culture flasks by vigorous shaking or treatment with trypsin. The cells were then washed three times with PBS, and labelled as described with 1 mCi ^{125}I to 2×10^7 cells. BCL were treated in an identical manner. Labelled cells were lysed in 1 ml 0·01 M Tris, 0·5% Triton X-100, 0·1 mM iodoacetamide, on ice for 15 min, and the lysate was passed over a 10 ml Sephadex G25 column.

Metabolic labeling of surface proteins with ^{35}S methionine

Maternal and fetal lymphocytes were metabolically labelled with ^{35}S methionine since it was not usually possible to obtain sufficient numbers of these cells for surface labelling. Cells were suspended in RPMI-1640 without methionine (Gibco), with 20% FCS, at a density of 5×10^6 per ml, and incubated at 37° for 1 hr. One-hundred μCi of ^{35}S methionine (Amersham International, Amersham, Bucks) per 5×10^6 cells was added, and the incubation continued overnight. Control lymphocytes were labelled by both methods (surface and metabolic labelling) to confirm that no differences could be observed in the surface antigens immunoprecipitated using the techniques available. After labelling, cells were washed once in PBS, then lysed in 0·5 ml of 50 mM Tris, 5 mM $MgCl_2$, 0·1 mM phenyl methyl sulphonyl fluoride (PMSF, Sigma), 0·5% Nonidet P-40 (NP-40, Sigma).

Immunoprecipitation

Aliquots (500 μl) of ^{125}I- and ^{35}S-labelled lysates were precleared prior to immunoprecipitation by the addition of 10 μg of rabbit anti-mouse immunoglobulin (Miles, Slough, Berks), followed by 50 μl of 10% formalin-fixed *Staphylococcus aureus* Cowan Strain 1 (SAC, Calbiochem, La Jolla, CA). Lysates were incubated at 4° for 1 hr, then centrifuged to remove the SAC. Five to ten μg of monoclonal antibody were then added to the

Table 1. Monoclonal antibodies (specificities and sources) used in this study

Monoclonal antibody	Antigen detected	Reference
JMB2	Intermediate filaments	McGee *et al.* (1982)
W6/32	HLA A, B, C	Barnstable *et al.* (1978) (gift of W. F. Bodmer)
MA2.1	HLA A2, B17	McMichael *et al.* (1980)
ME1	HLA B27, B7, B22	Ellis *et al.* (1982)
BBM.1	β_2 microglobulin	Brodsky *et al.* (1979) (gift of W. F. Bodmer)
MHM.5	HLA B, C	Ellis *et al.* (1985)
NDOG2	Placental alkaline phosphatase	Stirrat *et al.* (1983)
NA1/34	CD1	McMichael *et al.* (1979)
SG171	HLA DR	Goyert *et al.* (1982) (gift of S. M. Goyert)

lysates, and incubation at 4° continued for a further hour, followed by the addition of 50 μl of SAC. After a further hour the SAC was pelleted and washed three times in the appropriate lysis buffer. If the immunoprecipitate was required for SDS–PAGE analysis, Protein A sepharose CL 4B (Sigma) was used in preference to SAC. SAC-bound immunoprecipitates were treated with neuraminidase (Sigma Type V, 30 μl of 1 unit/ml at 37° for 4 hr) prior to elution for IEF gels.

SDS–polyacrylamide gel electrophoresis (PAGE)
Immunoprecipitated products were eluted from the SAC pellet, or from the Protein A sepharose, by boiling for 5 min with 30 μl of loading buffer (0·25 M Tris, pH 6·8, 4% SDS, 20% glycerol, 10% 2ME, 0·002% bromophenol blue). The supernatant was then loaded onto a standard 10% or 12% reducing gel (Laemmli, 1970) and run at a constant current of 30 mA. Gels were dried, then autoradiographed on Kodak XS film at −70°.

Isoelectric focusing (IEF)
SAC-bound immunoprecipitates were treated with neuraminidase for 4 hr, then eluted from the pellet by incubating at 50° for 30 min in 9 M urea, 2% NP40, 2% ampholines, 5% 2ME. For isoelectric focusing in slab gels, the method of van der Poel *et al.* (1983) was followed. The gels had a pH gradient of approximately 4–7, obtained by using a mixture of ampholines (3·5–10·0 and 5–7 in a ratio of 1 : 4), and were focused for 16 hr, then dried and autoradiographed.

RESULTS

Antigen expression on trophoblast (isolated and in tissue sections)

Staining serial sections of amniochorion with monoclonal antibodies to HLA antigens (W6/32,MA2.1,ME1) confirmed that antibodies to monomorphic determinants bound to cells in this tissue, but that antibodies to appropriate HLA A or B specificities did not, thus confirming earlier work (Redman *et al.*, 1984).

The average yield of trophoblast cells following stripping of the amniotic epithelium and enzyme digestion (data from 50 placentas) was $38 \pm 27 \times 10^6$, with an average viability of $88 \pm 6\%$. The populations were shown to be $78 \pm 15\%$ trophoblast by immunoperoxidase labelling of cytospin preparations with the monoclonal antibody JMB2 (McGee *et al.*, 1982), which in this situation reacts only with trophoblast. Labelling the cells in suspension with anti-HLA monoclonal antibodies confirmed the *in situ* results.

HLA types of donors and fetuses were determined by standard serological techniques. However, no positive typing results (Class I or Class II) could be obtained with trophoblast preparations, although complement-mediated cytotoxicity was observed using the IgG2a monoclonal antibody W6/32 to a dilution of 1/500.

Antigen expression on BeWo cells

Binding of monoclonal antibodies to HLA Class I was investigated on the choriocarcinoma cell line BeWo (Fig. 1). Between 70% and 90% of BeWo cells stained with each of BBM.1 (anti-β_2m),W6/32 (anti-Class I, monomorphic), and MHM.5 (anti-Class I, B and C locus). The pattern and level of binding were similar to that obtained on BCL. It was not possible to obtain a tissue type for BeWo (Class I or Class II) using standard serological techniques (see Discussion).

Analysis of antigen by SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

Immunoprecipitates from surface-labelled cells were analysed by SDS–PAGE. W6/32 and BBM.1 (but not the control antibodies NA134 and SG/171) precipitated β_2m and a polypeptide with an apparent molecular weight of 40,000 from the trophoblast preparations and from BeWo (Fig. 2). However, MHM.5, a monoclonal antibody that recognizes an epitope that is present on all HLA B and C antigens (Ellis *et al.*, 1985), and which we have shown to bind to BeWo, failed to precipitate either heavy chain or β_2m from trophoblast or from BeWo. It readily precipitates β_2m and a 45,000 MW heavy chain from BCL (Fig. 2). It was only possible to immunoprecipitate very small amounts of the 40,000 MW heavy chain (and β_2m) from ^{35}S methionine-labelled BeWo using W6/32 or BBM.1. Serial immunoprecipitations, using an irrelevant monoclonal antibody first, followed by W6/32 or BBM.1, demonstrated that binding to the 40,000 MW band was specific, and that the band did not represent an actin artifact.

Analysis of antigen by isoelectric focusing (IEF)

Immunoprecipitates from trophoblast and BeWo were analysed further by isoelectric focusing in slab gels, a technique that readily separates the polymorphic heavy chains of HLA A and B antigens, and β_2m (van der Poel *et al.*, 1983). The positions of most common HLA Class I antigens can be mapped using this system. In order to compare band positions between IEF gels, we use actin, which has a constant pI (4·9), as a marker. Actin appears as a contaminant in ^{35}S methionine-labelled lysates, and has a more acid pI than Class I heavy chains. The distance of each band from the origin is measured as a percentage of the distance between the actin band and the origin. The position of β_2m, which also has a constant pI (6·4), can then be used as an internal control, and our results show that it consistently gave a measurement of 37%. We can therefore positively identify most Class I A and B antigens by their relative positions on IEF gels, where all conditions and reagents are identical in each case.

Immunoprecipitates from trophoblast were focused alongside immunoprecipitates from the corresponding maternal and fetal lymphocytes of known HLA type. The trophoblast preparations failed consistently to show the typical multiple band pattern of HLA A, B and C antigens, within a pI range of 5·0–6·8, associated with β_2m. Instead, a band with a pI of 5·4 was obtained, from precipitates using W6/32 or BBM.1, and was never seen in control immunoprecipitates with two monoclonal antibodies of the same isotype as the anti-HLA reagents. This result was consistent in different trophoblast preparations in which there was no single shared HLA A, B or C antigen, maternal or fetal. Table 2 shows the results obtained from nine representative IEF gels. In some cases bands could not be positively identified, or were tentatively assigned the label HLA C. Results obtained with BCL are included to demonstrate the accuracy and reproducibility of the technique. Figure 3 shows autoradiographs of three IEF gels that demonstrate banding patterns obtained with immunoprecipitates from lymphocytes, trophoblast cells, BeWo and BCL. In approximately 30% of the

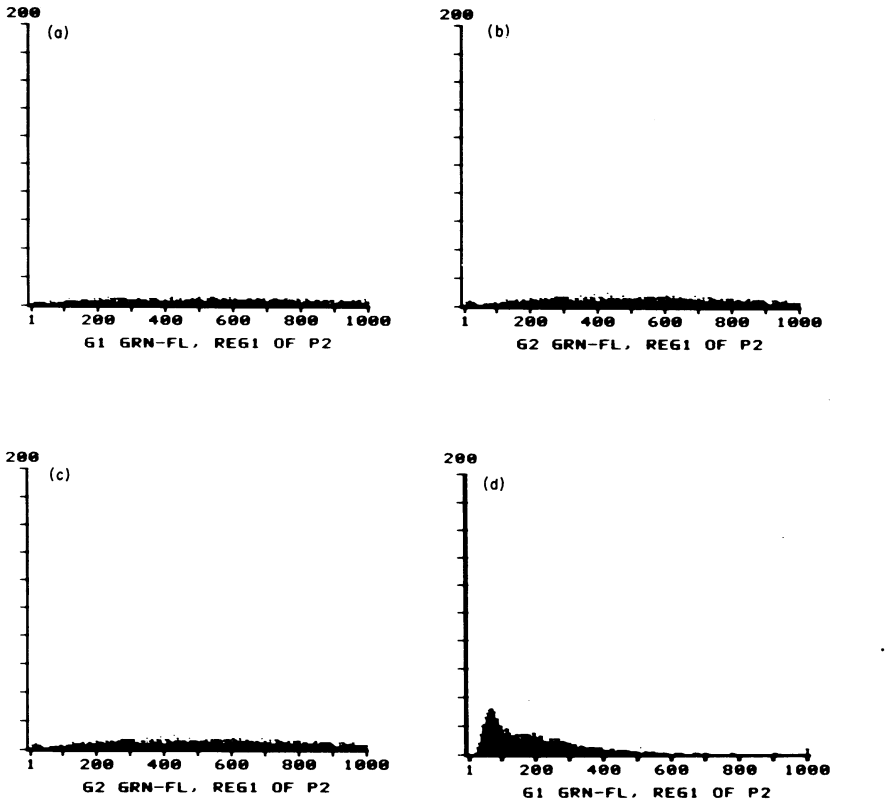

Figure 1. Fluorescence profiles of BeWo cells stained with (a) W6/32, (b) MHM.5, (c) BBM.1 and (d) NA1/34. Indirect immunofluorescence was carried out as described in the Materials and Methods. Stained cells were analysed on an Ortho Cytofluorograf. The *y* axis depicts frequency and the *x* axis fluorescence intensity on an arbitrary linear scale.

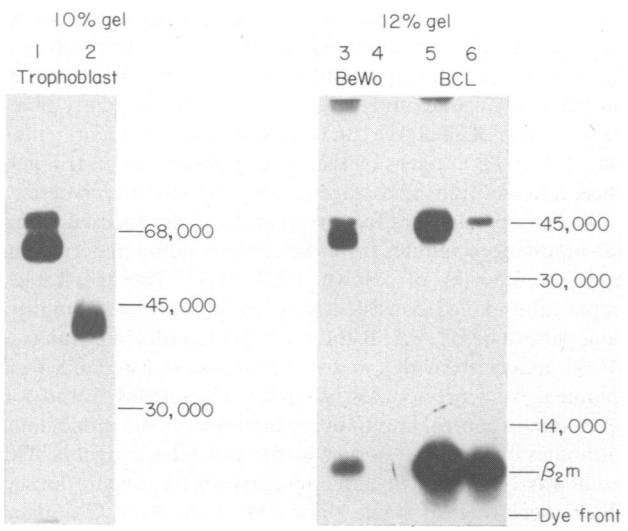

Figure 2. Autoradiograph from two SDS–polyacrylamide gels showing immunoprecipitated products from trophoblasts (Tracks 1 and 2), BeWo (Tracks 3 and 4), and BCL (Tracks 5 and 6) with BBM.1, anti-β_2m (Tracks 2, 3 and 5), NDOG2, anti-placental alkaline phosphatase (Track 1), and MHM.5, anti-HLA B, C (Tracks 4 and 6) on a 10% polyacrylamide gel (Tracks 1 and 2) and a 12% polyacrylamide gel (Tracks 3–6). The β_2m band is missing on Track 2 due to the low percentage of this gel. All cells were surface-labelled with ^{125}I.

trophoblast preparations examined the antigen was not detected by biochemical analysis, which may reflect a natural variation in the level of expression. In these cases HLA A and B were not detected. Results obtained with BeWo were very similar; the antigen was always detected on IEF gels, and focused in the same position as the trophoblast antigen (Table 2), i.e. 84%, with a pI of 5·4.

DISCUSSION

It was believed until recently that human trophoblast did not express MHC antigens as a mechanism for protection of the fetus from the potential threat of a maternal immune response (Goodfellow *et al.*, 1976). However, recent evidence shows that some forms of extravillous trophoblast express a non-polymorphic HLA Class I antigen (Redman *et al.*, 1984), although the precise nature and function of this is not clear. If such a molecule is expressed elsewhere on human tissue, it would be difficult to detect because its presence would be masked by classical Class I. As extravillous trophoblast appeared to be unique in expressing only this form of HLA, it provided the ideal starting material for this study.

It is important to exclude explanations for the results obtained, other than that of a novel Class I antigen. One such explanation is that normal HLA alone is being expressed, but at such low levels that antibodies to the polymorphic determinants

Table 2. Positions of bands on IEF gels

	Identity of band	% rel. to actin	Identity of band	% rel. to actin	Identity of band	% rel. to actin
Maternal lymphocytes	A2	25	β_2 microglobulin	37	β_2 microglobulin	37
	β_2 microglobulin	37	B70	43	A1	43
	A1	43	A3	76	HLA C?	52
	B57?	80	B7		A9/B60	72
	B27	86	HLA C?	88	B22	76
Fetal lymphocytes	A2	25	A2	25	β_2 microglobulin	37
	β_2 microglobulin	37	β_2 microglobulin	37	HLA C?	47
	A1	43	B70?	43	A9/B60	72
	B18	56	HLA C?	57	B44	79
	HLA C?	66	B51?	65		
	B57?	80	HLA C?	88		
Trophoblast cells	β_2 microglobulin	37	β_2 microglobulin	37	β_2 microglobulin	37
	trophoblast antigen	84	Trophoblast antigen	84	Trophoblast antigen	84
BeWo	β_2 microglobulin	37	β_2 microglobulin	37	β_2 microglobulin	37
	Trophblast antigen	84	Trophoblast antigen	84	Trophoblast antigen	84
B-lymphoblastoid cell line	A2	24	β_2 microglobulin	37	β_2 microglobulin	37
	β_2 microglobulin	37	A1	42	A1	42
	A32	47	B8	80	A10	48
	B22	75			B8	81
	B8	81				

The above table shows the positions of bands found on IEF gels (data from nine gels) in relation to the position of actin (expressed as a percentage). In each set, the maternal lymphocytes, fetal lymphocytes and trophoblast cells were from a single donor and were run together on a gel. The lymphocytes and BCL were labelled with ^{35}S methionine, and the trophoblast and BeWo cells were surface-labelled with ^{125}I. Immunoprecipitations were carried out using W6/32.

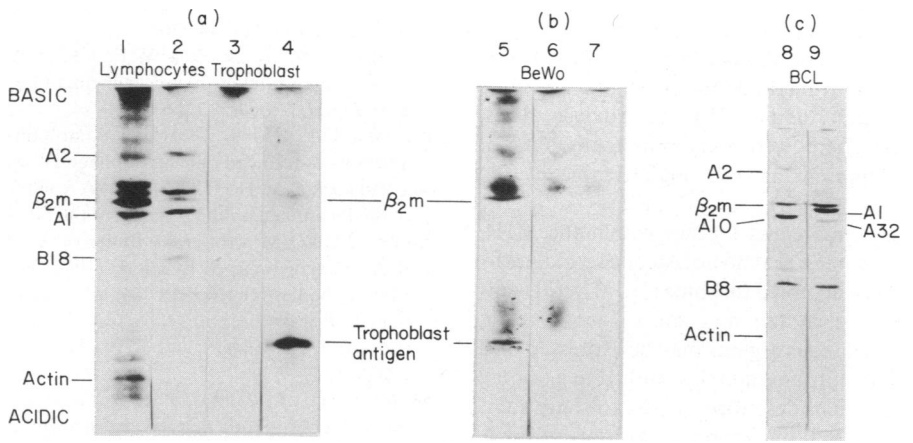

Figure 3. Autoradiographs from three isoelectric focusing gels comparing immunoprecipitates from trophoblast cells, BeWo cells, lymphocytes and BCL. Tracks 3 and 4 show immunoprecipitated products from a trophoblast cell preparation with (Track 4) W6/32, anti-HLA Class I, and (Track 3) NA1/34, anti-CD1. Track 1 shows products from the corresponding maternal lymphocytes, and Track 2 from the fetal lymphocytes immunoprecipitated with W6/32. Tracks 5, 6 and 7 show products from BeWo cells immunoprecipitated with BBM.1, anti-β_2m (Track 5), MHM.5, anti-HLA B, C (Track 6) and SG171, anti-HLA DR (Track 7). Tracks 8 and 9 show products from two BCLs immunoprecipitated with W6/32. The lymphocytes and BCL were labelled with ^{35}S methionine, the trophoblast and BeWo cells with ^{125}I.

present are not binding detectably. Our results of binding with monoclonal antibodies directed to monomorphic determinants of Class I, and to β_2m, both on trophoblast cells and the BeWo cell line, suggest that both β_2m and an associated heavy chain are expressed at easily detectable levels, comparable to those seen on lymphocytes and BCL. If it was normal HLA that we were detecting, it should be possible to obtain a tissue type for these cells, which was not the case.

Our results using SDS–PAGE to analyse immunoprecipitates from labelled cell lysates show not only that there is a high level of β_2m expression, but that the associated heavy chain has a lower molecular weight than normal Class I. The levels obtained

were clearly lower than those seen on BCL, but were still easily detectable (Fig. 2).

Results with the monoclonal antibody MHM.5 are difficult to explain. The antibody appears to bind to the cell surface of BeWo cells to exactly the same level as W6/32 and BBM.1. It does not, however, immunoprecipitate anything from the cell surface, although it readily immunoprecipitates β_2m and 45,000 MW heavy chains from control BCLs and lymphocytes (Fig. 2). It may be possible to explain this in terms of binding affinity; MHM.5 may fail to immunoprecipitate the Class I-like molecule from the surface of BeWo cells because its affinity for it is lower than its affinity for normal HLA B and C molecules. A similar phenomenon has been observed on some T-ALL cells (Orgad *et al.*, 1985) where extra HLA Class I antigens were observed which were precipitated by W6/32 but not by the monoclonal antibody 4E, which has a similar specificity to MHM.5.

Other explanations for the results could be that HLA C alone is expressed in large amounts, or that normal HLA A, B and C are transcribed, but are then truncated or modified in some way prior to surface expression. Our results with IEF of material from trophoblast cells and BeWo clearly show that the multiple heavy chain bands normally seen in association with β_2m are absent, and so rule out both of these explanations. Although we do not know the positions of most HLA C antigens on IEF gels, we know from tissue typing that there was no single shared HLA C antigen between the donors investigated, and yet we could see an invariant band in material immunoprecipitated from trophoblast cells and BeWo. Modified HLA A, B and C antigens would show a much greater reduction in molecular weight than that observed, if they were to lose all pI heterogeneity. The fact that the single band observed on IEF gels corresponds to the 40,000 MW observed on SDS–PAGE was demonstrated by using both methods to investigate the same immunoprecipitated sample, and by 2-D gel analysis. It is, however, possible that there were very small amounts of classical HLA Class I present as well, undetectable by the methods used.

The existence of multiple Class I genes within the MHC region of human chromosome 6 that do not encode serologically defined molecules has been accepted for some time. There is now mounting evidence in the mouse and rat to suggest that molecules encoded by analagous regions may be expressed on a variety of tissues. In many of the examples studied (e.g. mouse Qa, Tla, rat Pa) the molecules described appear to share some sequence with classical Class I, but are much less polymorphic, and often have a lower molecular weight, around 40,000 (Stroynowski *et al.*, 1985; Macpherson *et al.*, 1986).

The molecule that we describe seems to fit many of the criteria for a Class I molecule, yet its characteristics suggest that it does not belong in the HLA A, B or C series. We therefore propose that we have positively identified and characterized a human non-classical HLA Class I molecule. This, or similar molecules, may prove to be expressed on other adult or fetal tissue. Final proof that this is an MHC antigen will have to await gene sequencing and mapping.

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REFERENCES

ANDERSON D.J. & BERKOWITZ R.S. (1985) γ Interferon enhances expression of Class I MHC antigens in the weakly HLA+human choriocarcinoma cell line BeWo, but does not induce MHC expression in the HLA-choriocarcinoma cell line Jar. *J. Immunol.* **135,** 2498.

BARNSTABLE C.J., BODMER W.F., BROWN G., GALFRE G., MILSTEIN C. & WILLIAMS A.F. (1978) Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens. *Cell*, **14,** 9.

BRODSKY F.M., BODMER W.F. & PARAM P. (1979) Characterisation of a monoclonal anti $\beta_2$microglobulin antibody and its use in the genetic and biochemical analysis of MHC antigens. *Eur. J. Immunol.* **9,** 536.

ELLIS S.A., TAYLOR C., HILDRETH J.E.K. & MCMICHAEL A.J. (1985) An HLA Class I specific monoclonal antibody that fails to bind to all HLA-A antigens. *Human Immunol.* **13,** 13.

ELLIS S.A., TAYLOR C. & MCMICHAEL A.J. (1982) Recognition of HLA-B27 and related antigens by a monoclonal antibody. *Human Immunol.* **5,** 49.

GOODFELLOW P.N., BARNSTABLE C.J., BODMER W.F., SNARY D. & CRUMPTON M.J. (1976) Expression of HLA system antigens on placenta. *Transplantation*, **22,** 595.

GOYERT S.M., SHIVELY J.E. & SILVER J. (1982) Biochemical characterisation of a second family of human Ia molecules. HLA DS, equivalent to murine IA subregion molecules. *J. exp. Med.* **156,** 550.

HOOD L., STEINMETZ M. & GOODENOW R. (1982) Genes of the MHC. *Cell*, **28,** 685.

KAWATA M., SIZER K., SEKIYA S., PARNES J.R. & HERZENBERG L.A. (1984) Limitation of differential expression of HLA-A, B, C antigens on choriocarcinoma cell lines by mRNA for HLA heavy chain but not by β_2m. *Cancer Res.* **44,** 401.

KNOWLES R.M. & BODMER W.F. (1982) A monoclonal antibody recognising a human thymus leukemia-like antigen associated with β_2m. *Eur. J. Immunol.* **12,** 676.

LAEMMLI U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*, **227,** 680.

MACPHERSON T.A., HO H., KUNZ H.W. & GILL T.J. (1986) Localisation of the Pa antigen on the placenta of the rat. *Transplantation*, **41,** 392.

MCGEE J.O'D., MORTON J.A., BARBATIC C., BRADLEY J.F., FLEMING K.A., GOATE A.M. & BURNS J. (1982) Monoclonal antibodies to Mallory bodies/intermediate filaments and HLA (Class I) antigens in human liver disease. In: *Monoclonal Antibodies in Clinical Medicine* (Eds A. J. McMichael and J. W. Fabre) p. 431. Academic Press, London.

MCMICHAEL A.J., PARHAM P.R., RUST N. & BRODSKY F.M. (1980) A monoclonal antibody that recognises an antigenic determinant shared by HLA-A2 and -B17. *Human Immunol.* **1,** 121.

MCMICHAEL A.J., PILCH J.R., GALFRE G., MASON D.Y., FABRE J.W. & MILSTEIN C. (1979) A human thymocyte antigen defined by a hybrid myeloma monoclonal antibody. *Eur. J. Immunol.* **9,** 205.

ORGAD S., YANG S., GAZIT E., RELIAS V., ZAIZOV R., LYSON S. & YUNIS E.J. (1985) Expression of extra Class I MHC antigens on T cell ALL lymphoblasts. *Human Immunol.* **12,** 133.

PLOEGH H.L., ORR H.T. & STROMINGER J.L. (1981) MHC antigens: the human (HLA-A, -B, -C) and murine (H-2K,H-2D) Class I molecules. *Cell*, **24,** 287.

VAN DER POEL J.J., MOLDERS H., THOMPSON A. & PLOEGH H.L. (1983) Definition of four HLA A2 subtypes by CML typing and biochemical analysis. *Immunogenetics*, **17,** 609.

REDMAN C.W.G. MCMICHAEL A.J., STIRRAT G.M., SUNDERLAND C.A. & TING A. (1984) Class I MHC complex antigens on human extravillous trophoblast. *Immunology* **52,** 457.

STIRRAT G.M., SUNDERLAND C.A. & REDMAN C.W.G. (1983) Mono-

clonal antibodies in reproductive immunology—elucidation by monoclonal antibody techniques. In: *Obstetrics and Gynaecology Annual* (Ed. R. N. Wyn), Vol. 12, p. 43 Appleton-Century Crofts, Connecticut.

STROYNOWSKI I., FORMAN J., GOODENOW R.S., SCHIFFER S.G., MCMILLAN M., SHARROW S.O., SACHS D.H.& HOOD L. (1985) Expression and T cell recognition of hybrid antigens with amino-terminal domains encoded by Qa-2 region of MHC and carboxyl termini of transplantation antigens. *J. exp. Med.* **161,** 935.

TROWSDALE J., TRAVERS P., BODMER W.F. & PATILLO R.A. (1980) Expression of HLA-A, -B and -C and β_2m antigens in human choriocarcinoma cell lines *J. exp. Med.* **152,** 115.