Immunology 1988 **65** 93–99

Characterization of a 95,000 molecule on sheep leucocytes homologous to murine Pgp-1 and human CD44

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Accepted for publication 18 May 1988

SUMMARY

The phagocyte glycoprotein-1 (Pgp-1) antigen of mice is a 94,000 MW molecule with a wide tissue distribution, but no attributed function. We produced a monoclonal antibody (mAb) termed 25-32 which recognizes the Pgp-1 molecule of numerous mammalian species, including humans and sheep. Preclearing experiments with I42/5, a rat anti-mouse Pgp-1 mAb that cross-reacts with human Pgp-1, established the specificity of 25-32 for human and sheep Pgp-1. Moreover, an antibody recognizing human CD44, termed F10-44-2, also reacted with the same molecule as that recognized by 25-32 and I42/5, so establishing the co-identity of CD44 and Pgp-1. Within the sheep thymus, Pgp-1 was expressed most strongly by medullary thymocytes and stromal cells, and by small numbers of cells at the subcapsular cortex. Pgp-1 was expressed early in thymic ontogeny; all 35–40-day gestation fetal sheep thymocytes were intensely Pgp-1$^+$, but by 80 days the number of reactive thymocytes had decreased to adult levels. The expression of Pgp-1 on lymphocytes was markedly increased after stimulation with mitogens, or with phorbol esters and ionomycin. The highly conserved nature of Pgp-1 through evolution, its expression on virtually all cell types within the body, and its increased expression on rapidly dividing cells indicate that this molecule mediates an important function, possibly serving as a hormone or metabolite receptor.

INTRODUCTION

Pgp-1 in mice is a 94,000 MW polypeptide that exhibits a broad tissue distribution; most leucocyte cell types express this antigen, as do many non-lymphoid tissues such as brain, kidney and liver (Trowbridge *et al.*, 1982; Lesley & Trowbridge, 1982; Sutton *et al.*, 1986). However, a peculiar feature of Pgp-1 in mice is its absence from most thymocytes. Of those thymic cells that are reactive, a high proportion represent early stem cells (Lesley, Hyman & Schulte, 1985; Trowbridge *et al.*, 1985). Moreover, during fetal thymic development in mice, the number of Pgp-1$^+$ cells decreases from 80% to 90% at 13 days of gestation to as few as 5% at Day 19 (Lesley, Trotter & Hyman, 1985). An analogue of the Pgp-1 molecule has been identified recently in humans (Isacke *et al.*, 1986), but the expression of this molecule on thymocytes in man is different from that of mice, being 50–60% in contrast to the 5–10% of mouse thymocytes that express the molecule.

We report here on the identification of the Pgp-1 molecule in sheep, its molecular characterization and tissue distribution, its decrease in expression within the thymus during fetal development, and its increased expression on lymphocytes following mitogenic stimulation. In addition, co-identity between murine Pgp-1 and human CD44 was established using preclearing experiments with anti-Pgp-1 mAbs and anti-CD44.

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Abbreviations: BSA, bovine serum albumin; Con A, concanavalin A; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; IPP, ileal Peyer's patch; mAb, monoclonal antibody; PBL peripheral blood lymphocyte; PBS, phosphate-buffered saline; Pgp-1, phagocyte glycoprotein-1; PHA, phytohaemagglutinin; PMA, phorbol myristate acetate; RIA, radioimmunoassay; SAMIg, sheep anti-mouse immunoglobulin; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Production of mAb

BALB/c mice were obtained from the Walker and Eliza Hall Institute, Melbourne. Mice were immunized intraperitoneally with 2×10^7 sheep efferent lymphatic duct lymphocytes once a week over 4 weeks. A boost of 2×10^7 lymphocytes was given intravenously 4 days before fusion. Immune spleen cells were fused with NS-1 myeloma cells as described previously (Galfre *et al.*, 1977). Hybridomas were screened for activity against sheep lymphocytes by indirect radioimmunoassay (RIA). Specific hybridomas were cloned by limiting dilution and further characterized by immunohistology, immunofluorescence and RIA, as described elsewhere (Mackay *et al.*, 1985). Other mAbs used included a rat antibody I42/5, raised against mouse Pgp-1, which cross-reacts with human Pgp-1 (Trowbridge *et al.*, 1982);

Table 1. Reactivity of mAb 25-32 with sheep and human cells

Cell type	% stained cells*	
	Sheep	Human
Peripheral blood lymphocytes	>95	>95
Lymph node lymphocytes	90	ND†
Efferent lymph lymphocytes	>95	ND
Thymocytes	55	48
Ileal Peyer's patch cells	85	ND
Macrophages, monocytes, granulocytes	>95	>95

*Percentage staining was assessed by indirect immunofluorescence and flow cytometry. Figures represent data from at least six individuals.

†ND, not done.

F10-44-2, a mouse mAb reactive with a human leucocyte antigen of MW 95,000 (Dalchau, Kirkley & Fabre, 1980); 41-19 and 28-1, mouse mAbs which react with sheep MHC class I and class II antigens, respectively (Puri, Mackay & Brandon, 1985; Gogolin-Ewens *et al.*, 1985); and 44-38, an anti-sheep CD4 mAb (Maddox, Mackay & Brandon, 1985). Anti-human CD4 and MHC class II mAbs were purchased from Ortho Diagnostics.

Indirect radioimmunoassay

Fresh and glutaraldehyde-fixed sheep lymphocytes were used as targets for screening and testing hybridoma culture supernatants by an indirect binding radioimmunoassay (Morris & Williams, 1985) using rabbit anti-mouse $F(ab')_2$ (kindly provided by Dr A. F. Williams, William Dunn School of Pathology, Oxford University).

Immunohistochemistry

Antigens were localized on sections of frozen tissues using an indirect immunoperoxidase method with diaminobenzidine as substrate (Mackay *et al.*, 1985).

Cells and tissues

Peripheral blood lymphocytes (PBLs) from either sheep or humans were fractionated by Ficoll–Hypaque (Pharmacia) density centrifugation, or were identified in ammonium chloride-lysed blood using 0° and 90° light scatter and flow cytometry. Sheep lymphocytes were also collected from a cannulated efferent duct of the prescapular lymph node (Lascelles & Morris, 1961). Sheep tissues for immunohistology were obtained from penned sheep or an abattoir. Sheep alveolar macrophages were collected using lung lavage with PBS. Granulocytes were identified by their 0° and 90° light scatter on the fluorescence-activated cell sorter (FACS), when ammonium chloride-lysed blood was analysed.

Immunofluorescent staining and flow cytometry

Two to three times 10^6 cells in 50 μl of PBS/1% BSA were incubated with 50 μl of neat mAb tissue culture supernatant. After 20 min the cells were washed three times with PBS azide/1% BSA, and incubated for 20 min with FITC-conjugated sheep anti-mouse IgG (FITC-SAM, Silenus Laboratories, Mel-

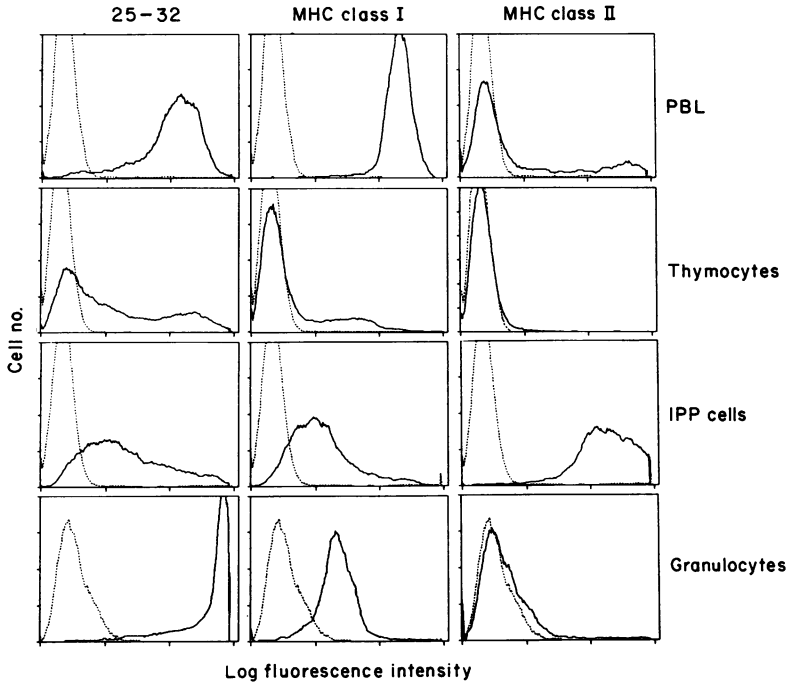

Figure 1. Analysis of sheep leucocytes stained with mAbs, using indirect immunofluorescence and flow cytometry. Cells were stained with either 25-32, 41-19 (MHC class I-specific) or 28-1 (mHC class II-specific), followed by FITC-SAM Ig (see the Materials and Methods). For each cell type, 10^4 cells were analysed, and propidium iodide was used to exclude dead cells. Panels show profiles for PBL, thymocytes, IPP cells and peripheral blood granulocytes, as indicated.

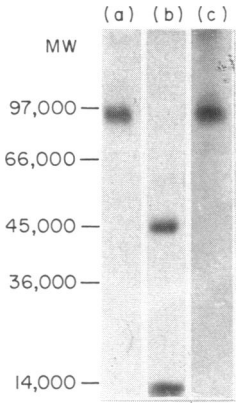

Figure 2. SDS–PAGE analysis of material immunoprecipitated by mAb 25-32 from a sheep lymphocyte lysate. (a) 25-32, reduced. (b) MHC class I, reduced. (c) 25-32, non-reduced.

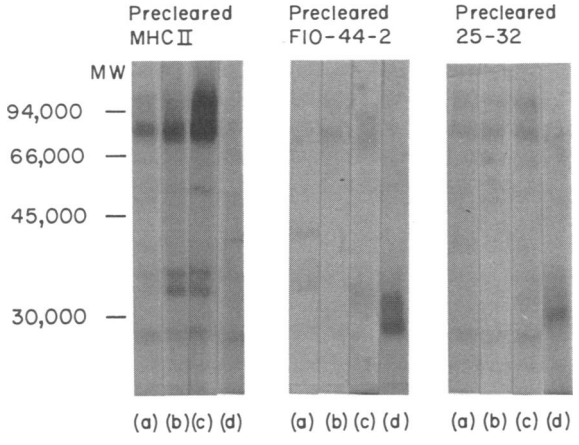

Figure 3. Co-identity between the molecules recognized by mAbs 25-32, I42/5 (anti-Pgp-1) and F10-44-2 (anti-CD44) from a human leucocyte lysate. RBC were removed from human peripheral blood by ammonium chloride lysis, and the remaining leucocytes were surface labelled with I^{125} by the lactoperoxidase method. Portions of the lysate were extensively precleared using either 25-32, F10-44-2 or an anti-MHC class II mAb. Lysates were then reacted with I42/5 (a), 25-32 (b), F10-44-2 (c) or anti-MHC class II (d). All tracts were run on a 10% polyacrylamide gel under reducing conditions.

bourne, Australia). After washing three times with PBS azide/1% BSA, cells were examined using a FACS, or were fixed and analysed at a later date.

Lactoperoxidase-catalysed cell surface iodination and immunoprecipitation

Cell surface antigens of lymphocytes were labelled with ^{125}I using a lactoperoxidase catalysed reaction (Goding, 1980). Washed viable cells were resuspended at 5×10^7 cells in 200 μl of PBS, and to the cell suspension were added 50 μl of lactoperoxidase (0·4 mg/ml, type V, Sigma, St Louis, MO) and 0·5 MCi ^{125}I (Amersham, Bucks, U.K.). Successive 10-μl pulses of H_2O_2 at the following concentrations were then added at 1-min intervals:

Table 2. Tissue distribution of the 25-32 antigen in sheep assessed by immunohistology

Tissue	Reactivity of mAb 25-32
Thymus	Medullary thymocytes and some medullary stromal cells. Outer cortical thymocytes, especially during fetal ontogeny
Lymph node	Connective tissue, all lymphocytes except some cells within germinal centers
Liver	Kupffer cells, connective tissue, sinusoidal lining cells; hepatocytes negative
Kidney	Tubular epithelial cells, connective tissue; glomeruli negative
Brain	White matter; blood vessel endothelium negative
Gut	Connective tissue, smooth muscle, epithelia, lymphocytes

0·3 mM, 1 mM, 3 mM and 9 mM. Cells were washed twice in 10 ml of ice-cold PBS and lysed in 1 ml of PBS containing 0·5% Nonidet P-40 (NP-40; BDH) for 60 min at 4°. Nuclei and debris were removed from the lysate by centrifugation at 6000 *g* for 10 min a Beckman Microfuge.

Lymphocyte lysates were precleared of any material which bound non-specifically to protein A–Sepharose (Pharmacia). Samples of the lysate (2×10^6 c.p.m.) were added to tubes containing 200 μl of mAb tissue culture superantant. Lysates were incubated for 2–4 hr with mAb, after which 50 μl of a 50% (v/v) suspension of protein A–Sepharose were added. After incubation for 2 hr at 4° on an orbital rotator, the Sepharose beads were washed four times in cold 0·5% NP-40 in PBS, and immunoprecipitated material attached to the beads was analysed by SDS–PAGE (Laemmli, 1970).

Mitogen stimulation of sheep lymphocytes

For *in vitro* stimulation with mitogens, lymphocytes were obtained from peripheral blood or efferent lymph and were suspended at 10^6 cells/ml in DME media supplemented with 10% FCS, 2 mM L-glutamine, $2{\cdot}5 \times 10^{-5}$ M 2-mercaptoethanol and penicillin-streptomycin (50 IU/ml and 50 μg/ml, respectively; Flow Laboratories, Sydney, Australia). Cells were stimulated with either 10 μg/ml of concanavalin A (Con A; Sigma) or 25 μg/ml of phytohaemagglutinin (PHA; Sigma) at 37° in a 5% CO_2 atmosphere. The mitogens used at these concentrations gave maximal stimulation, as determined by tritiated thymidine incorporation. Mitogen activation for phenotypic analysis was performed in 24-well Linbro plates. After various intervals, cells were washed, stained with mAb and analysed by a FACS, using propidium iodide to exclude dead cells. Lymphocytes were also stimulated with a combination of 50 ng/ml phorbol myristate acetate (PMA) (Sigma) and 300 ng/ml ionomycin (Calbiochem, Behring Diagnostics, La Jolla, CA).

RESULTS

Identity of a molecule on sheep lymphocytes reactive with mAb 25–32

MAb 25-32 reacted with virtually all sheep peripheral lymphocytes, whether from peripheral blood, efferent lymph or lymph

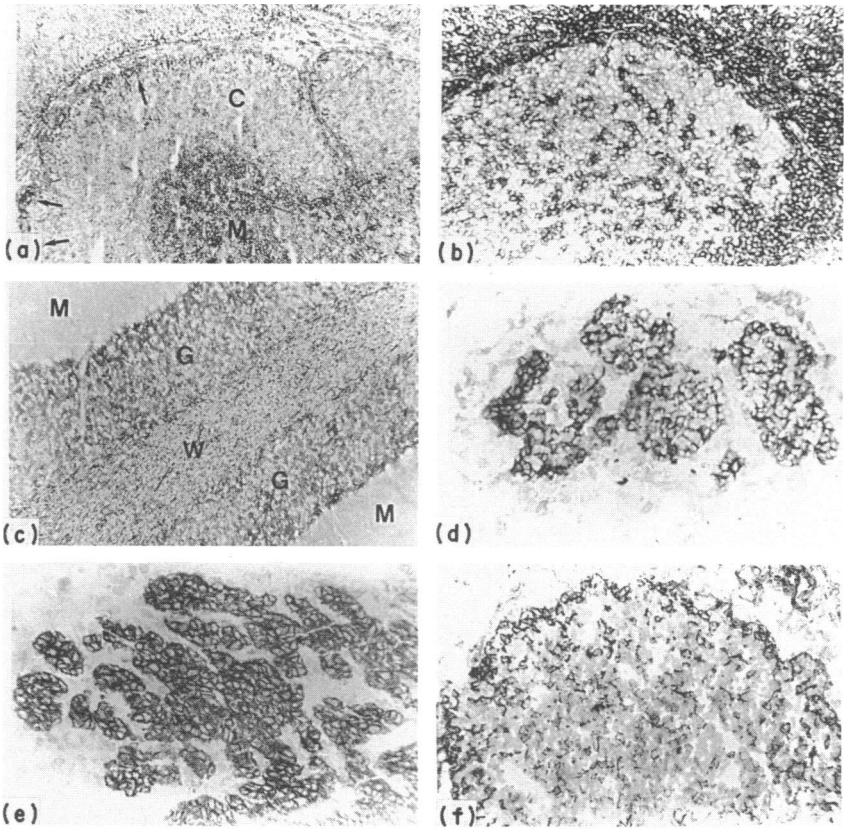

Figure 4. Immunoperoxidase staining of adult and fetal sheep tissues with mAb 25-32. (a) Thymus, × 70, showing reactivity with the medulla (M) and subcapsular cortex (arrows). The remaining cortex (C) stained weakly. (b) Lymph node, × 126, showing a germinal centre with mostly non-reactive cells; T-cell areas surrounding the germinal centre contained numerous reactive cells. (c) Cerebellum, × 70, showing the different zones with varying staining patterns; the outer molecular layer (M) was weakly stained, whereas the granular layer (G) and the central core of white matter (W) were intensely stained. (d) Day 35 fetal thymus, × 168, showing reactivity with the great majority of cells. (e) Day 40 fetal thymus, × 168. (f) 45 fetal thymus, × 168, showing staining localized to the subcapsular region and to scattered epithelial cells.

node. Table 1 and Fig. 1 show the reactivity of mAb 25-32 with various leucocyte cell types of sheep, as determined by indirect immunofluorescence and flow cytometry. Whereas most peripheral lymphocytes expressed high levels of the antigen reactive with 25-32, only a proportion of thymocytes (55%) were positive. Two-colour immunofluorescence analysis of sheep lymphocytes stained with 25-32-PE and anti-sheep Ig-FITC demonstrated that sIg^- lymphocytes expressed higher levels of the antigen reactive with 25-32 than did sIg^+ lymphocytes (data not shown).

Non-lymphoid cells from sheep were also tested for reactivity with 25-32. Alveolar macrophages gave the most intense staining (data not shown), and all granulocytes were $25\text{-}32^+$, with a staining intensity stronger than that observed for sheep T cells.

The molecular nature of the 25-32-reactive antigen was assessed by SDS–PAGE (Fig. 2). From lymphocytes, the material immunoprecipitated by 25-32 migrated at 95,000 MW, under both reducing and non-reducing conditions.

A surprising feature of mAb 25-32 was its cross-reactivity with numerous mammalian species, including humans (see below). We analysed the reactivity of mAb 25-32 against human leucocytes, and showed that FACS profiles and the percentages of positively stained leucocytes very much resembled that found in sheep (Table 1). Macrophages, granulocytes and T cells expressed the highest levels, whereas only 48% of thymocytes were stained.

Co-identity between the 25-32 molecule, murine Pgp-1 and human CD44

The distribution of 25-32-reactive cells in sheep and humans described above suggested similarities between this antigen and the mouse Pgp-1 molecule. In addition, given the high density of the 25-32 antigen on human T cells, it appeared likely that mAbs would have already been described for this structure. We therefore investigated whether the reactive antigen was in fact the homologue of murine Pgp-1, and/or a number of previously described human leucocyte antigens. As shown in Fig. 2, material immunoprecipitated by 25-32 from sheep lymphocytes migrated on SDS–PAGE with a mobility corresponding to 94,000 MW under reducing conditions. Material immunoprecipitated from human leucocyte lysates by 25-32, however, showed more heterogeneity (Fig. 3). This was probably due to the numbers of different cell types that were used to prepare the human cell lysate, since peripheral blood leucocytes were used,

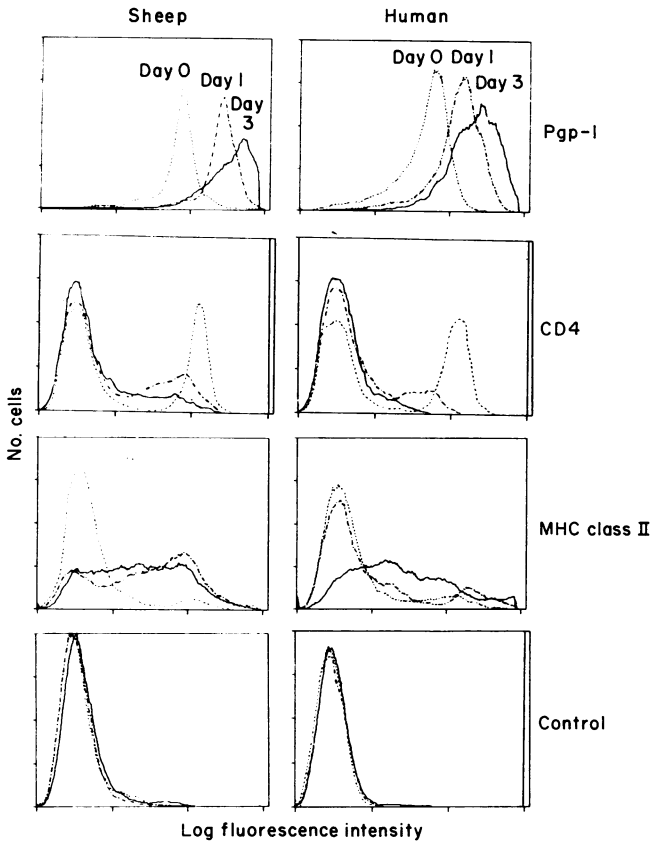

Figure 5. Enhanced expression of CD44 on sheep and human lymphocytes following mitogenic stimulation. Lymphocytes were stimulated with PMA/ionomycin for 0, 1 and 3 days and were stained with mAb followed by sheep anti-mouse Ig-FITC, prior to FACS analysis. 25-32 was used for analysis of both human and sheep cells, whereas different anti-CD4 and MHC class II mAbs were used to distinguish the respective sheep and human molecules.

which included lymphocytes, granulocytes and monocytes. We have not yet examined whether these leucocyte types express different MW forms of the 25-32 antigen, nor if sheep granulocytes express a lower MW form than the 95,000 MW structure immunoprecipitated from lymphocytes.

The formal identification of these antigens in sheep and humans as Pgp-1 was established using the species cross-reactivity of a rat anti-mouse Pgp-1-specific mAb, I42/5, which reacts with human Pgp-1 (Trowbridge *et al.*, 1982). Sequential immunoprecipitation of human lysates demonstrated that 25-32 was able to preclear Pgp-1 from the lysate (Fig. 3). These sequential immunoprecipitation studies, and the similarity in molecular size of the antigen reactive with 25-32 in sheep and the Pgp-1 molecules in mice and humans, established that the mAb 25-32 is directed to the Pgp-1 molecule in sheep and humans.

Of the human leucocyte antigens described in the literature, CD44 closely resembled the 25-32-reactive antigen. CD44 has been described as a brain/macrophage/leucocyte antigen of MW 100,000 MW (Dalchau *et al.*, 1980; McKenzie, Dalchau & Fabre, 1982). When human lymphocyte lysates were precleared of CD44 using the mAb F10-44-2, no material could be immunoprecipitated using mAb 25-32 (Fig. 3). The co-identity between the antigens recognized by these two antibodies was also indicated by similar reactivities and FACS profiles for the two antibodies on human lymphocytes and thymocytes (see below). However, 25-32 and F10-44-2 must recognize different epitopes on the one molecule, since F10-44-2 was unreactive with sheep cells.

Distribution of Pgp-1/CD44 within adult and fetal sheep tissues

MAb 25-32 was reactive with cells from various sheep tissues, as shown by immunohistological staining on frozen sections (Table 2). Within lymph nodes, 25-32 stained virtually all cells except those within the germinal centres (Fig. 4). Within the thymus, positive staining was localized to the medulla and to areas at the subcapsular cortex. Staining in the medulla often showed a dendritic pattern, signifying reactivity with stromal elements, although medullary thymocytes were also stained. This pattern was suggestive of reactivity with macrophages and dendritic cells within the medulla, although reactivity with medullary epithelium could not be excluded. Sections of cerebellum stained with 25-32 revealed strong reactivity with the white matter (Fig. 4).

We next examined the distribution of Pgp-1$^+$ cells within the sheep thymus during fetal development using immunoperoxidase staining of frozen tissue sections. At 32-35 days, the fetal sheep thymus consists of small islets of cells, most of which are negative for T-cell antigens (Mackay, Maddox & Brandon, 1986). Thymocytes at this stage have a cell surface phenotype that corresponds with that of a cell type located predominantly within the outer cortex of adult thymus, consistent with the notion that immature, thymic progenitor cells are concentrated within the outer cortical region of the thymus. When sections of 35-day-old and 40-day-old fetal thymus were reacted with the 25-32 mAb, virtually all cells were positive (Fig. 4), including both thymocytes and stromal elements. At 40–43 days of gestation, expression of the CD4, CD8 and CD1 antigens becomes apparent on the majority of thymocytes (Mackay *et al.*, 1986), indicating the emergence of cells with the phenotype of the typical cortical thymocyte; at this stage, Pgp-1 was localized to outer cortical regions, and connective tissue associated with the capsule and septa. At 60–80 days of gestation the staining pattern resembled that of post-natal thymus, with medullary areas showing strong staining for Pgp-1.

Increased expression of Pgp-1/CD44 on activated lymphocytes

Sheep or human lymphocytes were stimulated with either Con A PHA or PMA/ionomycin, and the kinetics of Pgp-1 expression by the stimulated cells was monitored using immunofluorescence and flow cytometry.

As shown in Fig. 5, both sheep and human lymphocytes expressed much higher amounts of Pgp-1 after stimulation with either mitogens or PMA/ionomycin, and enhanced expression was apparent by 24 hr. Maximum levels of Pgp-1 expression were observed by 3 days, after which levels remained relatively unchanged. PMA/ionomycin was more effective in promoting Pgp-1 expression on lymphocytes than was Con A or PHA. PMA alone had only a minimal effect on Pgp-1 expression. Stimulation of lymphocytes with PMA-ionomycin caused a marked decrease in the levels of CD4 expression for both human and sheep lymphocytes. This phenomenon has been observed before for human CD4$^+$ cells (Weyand, Goronzy & Fatham, 1987), and we now show the same result for sheep T cells stimulated with PMA.

Based on the above results, we examined whether anti-Pgp-1 mAb inhibited blastogenesis or cell division. Sheep efferent lymph lymphocytes were cultured in the presence of optimal amounts of either PHA, Con A or PMA/ionomycin, with or without various concentrations of purified 25-32 mAb (up to 50 μg/ml); the presence of 25-32 in the cultures had no effect on cell division, as determined by incorporation of tritiated thymidine (data not shown). 25-32 mAb had no stimulatory effect on sheep cells, either by itself or in the presence of PMA.

Species specificity of 25-32

As noted above, mAb 25-32 was found to react with human leucocytes. MAb 25-32 was tested for its reactivity on tissue sections of thymus and lymph node from numerous other species. This antibody was reactive with leucocytes from all large mammals tested, including goat, ox, pig and horse. In all of these species, 25-32-stained cells with an intensity similar to that observed for sheep or human tissues. No reactivity was observed with leucocytes from chicken, rat or mouse. Of interest was the reactivity on equine thymus, in that almost all cortical thymocytes, as well as medullary thymocytes, were intensely stained (data not shown). The broad species reactivity of the 25-32 mAb indicates that it recognizes a Pgp-1 determinant which has been highly conserved in mammalian evolution. However, mAb F10-44-2 (anti-CD44), which reacts with the same leucocyte molecule on human cells as does 25-32, was unreactive with sheep cells.

DISCUSSION

We describe here the identification and characterization in sheep and humans of a cell surface molecule reactive with a mAb 25-32 raised against sheep leucocytes. The identity of Pgp-1 as the structure reactive with mAb 25-32 was based on several observations. First, the reactivity of 25-32 with sheep macrophages, granulocytes, T cells and the majority of B cells. Second, the unique staining pattern on adult and fetal thymus. Third, the similarity in molecular size between mouse Pgp-1 and the human and sheep molecules recognized by 25-32. Formal proof that 25-32 was in fact reactive with human and sheep Pgp-1 was provided by sequential immunoprecipitation of human lysates with 25-32 and a Pgp-1-specific mAb, 142/5, which recognizes both the murine and human molecules. Also, a mAb to human CD44, termed F10-44-2, reacted with the molecule recognized by 25-32, so establishing the co-identity of CD44 and Pgp-1.

Despite the identification of F10-44-2 and 25-32 as mAbs recognizing human Pgp-1, some discrepancies exist between the reactivities of these two mAbs, and a third mAb described as specific for human Pgp-1 (Isacke *et al.*, 1986). This latter antibody (E1/2), unlike F10-44-2 and 25-32, is reported to be unreactive with brain. Moreover, both F10-44-2 (Dalchau *et al.*, 1980) and 25-32 stain human granulocytes more intensely than peripheral T cells, in contrast to E1/2. The basis for these differences is not known. The three antibodies may react with entirely different epitopes on human Pgp-1, which show differential expression. That all three mAbs react with human Pgp-1 is very likely, since E1/2 (Isacke *et al.*, 1986), and now F10-44-2 and 25-32, are able to preclear Pgp-1 from human lysates. Moreover, human Pgp-1 (defined initially by the cross-reactivity of a rat anti-mouse Pgp-1 mAb for the human molecule) exhibits a peptide map identical to the antigen immunoprecipitated by E1/2 (Isacke *et al.*, 1986) or 25-32 (G.L. Wijffels, unpublished data). Despite this, the reactivity of both 25-32 and F10-44-2 on human tissues very much resembles that reported for E1/2. Within the human thymus, medullary thymocytes express the highest levels of Pgp-1, whereas in mice medullary thymocytes are largely negative.

The reactivity of mAb 25-32 with Pgp-1 of numerous mammalian species indicates a remarkable degree of conservation of this antigen during evolution. Most mammalian lymphocyte antigens have been maintained through evolution with respect to their structure, function and tissue distribution (Williams & Barclay, 1986), but the conservation of an antigen to the degree that wide species cross-reactivity occurs is most unusual. The highly conserved nature of mammalian Pgp-1 is suggested also by pronounced similarities in the peptide map between the murine and human molecules (G. L. Wijffels, unpublished data). On the other hand, the expression of Pgp-1 within the thymus appears to vary between species, since only 5–10% of mouse thymocytes express Pgp-1, whereas 40–60% of sheep thymocytes and 50–60% of human thymocytes do so. There may be authentic species differences in Pgp-1 expression but quantitative factors such as surface density differences between species, or affinity differences between various Pgp-1 mAb, might explain why FACS enumeration of Pgp-1$^+$ thymocytes within several species has yielded such disparate estimates. However, cell surface antigens may exhibit different tissue distributions between species, since Thy-1 is expressed within lymphoid tissues of rodents but not humans (Reif & Allen, 1964; Dalchau & Fabre, 1979).

The Pgp-1 molecule in mice has been claimed recently to selectively define the subpopulation of T cells that have encountered foreign antigen (Budd, Cerottini & McDonald, 1987a, b). Most peripheral T cells were shown to lack Pgp-1, and T cells which were primed by deliberate immunization were found almost exclusively within the Pgp-1$^+$ subpopulation. Taken at face value the distribution of Pgp-1 on sheep PBL or CD44 on human PBL does not accord well with these murine experiments. Sheep PBL are quantitatively and uniformly Pgp-1$^+$ (Fig. 1). Moreover, virtually all lymphocytes within blood or lymph from fetuses are Pgp-1$^+$, and display a similar staining intensity to adult lymphocytes stained for Pgp-1 (C. R. Mackay, unpublished data). It therefore seems most unlikely that the qualitative expression of Pgp-1 in sheep or human distinguishes virgin and memory T cells. However, a pronounced increase in the level of expression of Pgp-1 on most cells of sheep PBL resulted from activation of cells either by PHA or by PMA and ionocycin (Fig. 5). Thus, in sheep as well as in mouse (Lynch, Chaudhri & Ceredig, 1986) a profoundly increased level of Pgp-1 expression seems to accompany the process of cellular activation, *in vitro* at least. In sheep or humans, the quantitative expression of Pgp-1 may identify subsets of T cells or B cells with differing immune capacity. Studies in mice showed that stimulation of the Pgp-1$^+$ subset of T cells by mitogens yielded vastly increased production of interferon-γ, compared with the Pgp-1$^-$ subset (Budd, Cerottini & McDonald, 1987c). It is possible that the Pgp-1^{high} T cells in sheep or humans may be equivalent to the Pgp-1$^+$ subset in mice, with similar immunologic history and function.

ACKNOWLEDGMENTS

We thank Mrs Carolyn Harley for typing the manuscript, and Dr Ian Trowbridge for supplying the 142/5 hybridoma cell line.

This work was supported by the Australian Wool Research Trust Fund.

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