Monoclonal antibodies that recognize different membrane proteins that are deficient in Rh_{null} human erythrocytes

One group of antibodies reacts with a variety of cells and tissues whereas the other group is erythroid-specific

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1. Rh_{null} human erythrocytes lack all of the antigens of the Rh and LW blood group systems and have abnormal shape and an increased osmotic fragility. In this paper two murine monoclonal antibodies raised against intact human erythrocytes were used to investigate further the abnormalities in these cells. BRIC 125 reacts weakly with Rh_{null} erythrocytes and BRIC 69 does not react at all. The results showed that BRIC 125 reacts with a component of M_r 47 000–52 000 which has a substantial content of *N*-glycans. In contrast, BRIC 69 reacted with a band of M_r 31 000 together with a very diffuse band of M_r 35 000–52 000. Treatment of BRIC 69 immunoprecipitates with endoglycosidase F/peptidyl-*N*-glycosidase F resulted in the loss of both BRIC 69 reactive components and the appearance of a new band of M_r similar to that of the Rh(D) polypeptide. 2. BRIC 125 had a broad reactivity with cells in peripheral blood, whereas the reactivity of BRIC 69 was confined to erythrocytes. BRIC 125, but not BRIC 69, reacted with human kidney tissue and bound to endothelium in peritubular capillaries, arteries and veins as well as the epithelial tissue of distal tubules. BRIC 125 stained haemopoietic cells, foetal hepatocytes and megakaryocytes in foetal liver and sinusoidal cells, hepatocytes and portal tracts in adult liver. In contrast, BRIC 69 reactivity was confined to haemopoietic cells in foetal liver. The BRIC 125 epitope has a wide tissue distribution, suggesting the occurrence of a related group of polypeptides which have a general functional role on cell surfaces. 3. Rh_{null} erythrocytes are deficient in at least four different membrane polypeptides.

INTRODUCTION

The antigens of the Rh blood group system are of great importance in clinical haematology. These antigens, particularly the Rh(D) antigen, are highly immunogenic in man, giving rise to difficulties in blood transfusion therapy and causing haemolytic disease of the newborn (see Mollinson, 1983). Despite their importance, the structure of these antigenic determinants remains unknown. Recent studies suggest that the Rh(D) antigen is part of a transmembrane polypeptide of M_r approx. 30 000, which is unusual in that it does not appear to be glycosylated (Moore *et al.*, 1982; Gahmberg, 1982, 1983). The polypeptide is associated with the membrane skeleton of the red cell (Ridgwell *et al.*, 1984; Gahmberg & Karhi, 1984).

All of the antigens of the Rh blood group system are absent from red cells of individuals with Rh_{null} syndrome. Red cells from individuals with this syndrome have abnormal shape and have been reported to have abnormal cation permeability (Ballas *et al.*, 1984). Rh_{null} syndrome is associated with mild compensated haemolytic anaemia (Sturgeon, 1970). Rh_{null} red cells appear to lack the Rh(D) polypeptide and an additional poly-

peptide of M_r 34 000 recognized by a mouse monoclonal antibody (LICR/LON R6A) (Ridgwell *et al.*, 1983). These cells also lack antigens of the LW blood group system, which appear to be carbohydrate-dependent antigens located on a glycoprotein of M_r 40 000–47 000 (Mallinson *et al.*, 1986).

In this paper we describe several murine monoclonal antibodies which react weakly, or not at all, with Rh_{null} red cells. The red cell membrane polypeptides reacting with these antibodies have been defined. The epitopes recognized by one group of antibodies appear to be erythroid-specific. The other group of antibodies defines a previously undescribed glycoprotein. The antibodies that recognize this glycoprotein react with all other peripheral blood cells and with kidney and liver. These results suggest that this molecule has a general functional role in cells and tissues.

MATERIALS AND METHODS

Normal erythrocytes and human anti-Fy^a serum (Gou) were available at the South Western Regional Blood Transfusion Centre, Bristol. Blood from two

Abbreviations used: PBS, phosphate-buffered saline, pH 7.4 (Dulbecco A; Oxoid, Basingstoke, Hants., U.K.); PMSF, phenylmethanesulphonyl fluoride; Endo F, endoglycosidase F/peptidyl *N*-glycosidase F.

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Rh_{null} donors (regulator type) was supplied by Mr. E. B. McDonald and Dr. P. Harden (Red Cross Blood Transfusion Service, Brisbane, Australia; donor Y.T.) and by Mr. M. Bruce and G. S. Gabra (Glasgow and West of Scotland Blood Transfusion Service, Carlisle; donor S.M.; Gabra *et al.*, 1987). Erythrocytes from the LW(a-b-) donor (Mrs. Big) were supplied by Dr. B. P. L. Moore, Canadian Red Cross Blood Transfusion Service, Toronto, Ontario, Canada. Erythrocytes from the Fy(a-b-) donor (J.B.) were supplied by Mrs. M. Leak, South London Regional Transfusion Centre, Tooting, London. Human monoclonal anti-D culture supernatant was provided by Dr. B. Kumpel (U.K. Transplant Service, Bristol). Murine monoclonal antibody BS56 (anti-LWab) was supplied by Dr. H. Sonneborn, Biotest-Serum-Institut G.m.b.H., Frankfurt-am-Main, Federal Republic of Germany. Murine monoclonal antibody LICR/LON (R6A) was from Dr. P. A. W. Edwards (Department of Pathology, University of Cambridge). Murine monoclonal antibodies NBTS/BRIC 32 (BRIC 32), NBTS/BRIC 69 (BRIC 69), NBTS/BRIC 125 (BRIC 125) and NBTS/BRIC 126 (BRIC 126) were cloned from fusions performed using spleen cells from Balb/c mice given intraperitoneal injections of human erythrocytes (100 µl of a 50% suspension of cells in PBS, pH 7.3). BRIC 32 resulted from four injections at intervals of 14 days and 7 days respectively, and BRIC 69 from eight injections over a period of 8 months. BRIC 125 and BRIC 126 came from the same fusion and resulted from three injections over a period of 3½ months. In all cases fusion was performed 3 days after the final injection. The methods for cell culture and cloning were as described by Parsons *et al.* (1982), except that culture media were supplemented with 10% foetal bovine serum ['Myoclone'; Gibco (Europe)] in place of human serum. BRIC 32, BRIC 69, BRIC 125 and BRIC 126 were all of the IgG class. BRIC 69 was IgG₁, and BRIC 125 and BRIC 126 were IgG_{2b} (method of McDougal *et al.*, 1983). The subclass of BRIC 32 could not be determined. Standard serological techniques were used. For indirect agglutination tests polyspecific anti-mouse IgG was used as a second antibody. Treatment of intact erythrocytes with neuraminidase or with Pronase was as described (Anstee *et al.*, 1979; Judson & Anstee, 1977). SDS/polyacrylamide gel electrophoresis was carried out using the discontinuous buffer system of Laemmli (1970). Immunoblotting was as described in Mallinson *et al.* (1986), except that 5% bovine milk powder was used as the blocking agent.

All cell lines were maintained in Dulbecco's modified

Eagle's medium supplemented with 10% foetal bovine serum. Indirect immunofluorescence on cell lines and fresh blood cells separated on lymphocyte separation medium (Flow Laboratories) was as described (Parsons *et al.*, 1987). In some cases the immunofluorescence results were confirmed by flow cytometry using a Coulter EPICS C. Lymphoblastoid cell lines were created from peripheral blood lymphocytes of normal donors and the two Rh_{null} donors as described (Doyle *et al.*, 1985).

Immunoprecipitation was carried out essentially as in Moore *et al.* (1982). When BRIC 32 and BRIC 69 were used, the cells were also incubated with rabbit anti-(mouse IgG) (Dakopatts Z109) after attachment of the monoclonal antibody. Immunoprecipitation from erythrocyte skeletons was carried out essentially as described by Ridgwell *et al.* (1984).

Endo F digestion of erythrocytes prior to immunoprecipitation was carried out as described (Tanner *et al.*, 1988). Endo F digestion of solubilized immune complexes was performed as follows: immune complexes were boiled with 0.5% SDS, 2 mM-EDTA and 2 mM-PMSF, and then an equal volume of 200 mM-Na₂HPO₄, 100 mM-EDTA, 1% (v/v) Nonidet P-40, 2% 2-mercaptoethanol, 2 mM-PMSF, 10 mM-*o*-phenanthroline, pH 6.0, was added prior to addition of Endo F (2–5 µl) and incubation at 37 °C for 16 h.

Immunohistology on renal tissue was carried out as described by Evans *et al.* (1985).

RESULTS AND DISCUSSION

Specificity of monoclonal antibodies BRIC 125, BRIC 126 and BRIC 32

Three murine monoclonal antibodies (BRIC 125, BRIC 126 and BRIC 32) raised to intact human erythrocytes reacted more weakly with erythrocytes of the Rh_{null} phenotype than with normal erythrocytes. In the experiments described all these antibodies were used, but since both BRIC 125, BRIC 126 and BRIC 32 show the same properties, data for BRIC 125 only is given, except where indicated. Table 1 shows that although BRIC 125 gave weak reactions with untreated Rh_{null} cells, stronger reactions were obtained when the cells were treated with Pronase.

BRIC 125 reactive components in normal erythrocytes

Immunoprecipitation using BRIC 125 and radioiodinated normal Rh(D) positive or Rh(D) negative erythrocytes revealed two diffusely migrating labelled

Table 1. Specificity of monoclonal antibodies BRIC 125 and BRIC 69

Treatments: U, untreated; P, Pronase-treated; S, sialidase-treated; nt, not tested. A strong positive reaction is indicated by +, a weak positive reaction by w and a negative reaction by –.

Monoclonal antibody	Agglutination method	Treatment ...	Normal			Rh _{null}		LW(a-b-) U	Fy(a-b-) U
			U	P	S	U	P		
BRIC 125	Direct		+	+	+	–	+	+	+
	Indirect		nt	nt	nt	w	+	+	+
BRIC 69	Direct		–	+	+	–	–	–	–
	Indirect		+	nt	nt	–	–	+	+

Fig. 1. BRIC 125: autoradiographs of immunoprecipitates from radioiodinated intact erythrocytes, membrane skeletons and Endo F-treated intact erythrocytes and of Endo F-treated immune complexes

All tracks show autoradiographs of immunoprecipitates using the following antibodies and erythrocytes/membrane skeletons of different phenotypes. (a) Human monoclonal anti-D with cDE erythrocytes; (b) human monoclonal anti-D with Rh_{null} erythrocytes; (c) BRIC 125 with cDE erythrocytes; (d) BRIC 125 with Rh_{null} erythrocytes; (e) BRIC 125 with membrane skeletons from cDE erythrocytes; (f) BRIC 125 with cDE erythrocytes; (g) human monoclonal anti-D from cDE erythrocytes; (h) human monoclonal anti-D from Endo F-treated cDE erythrocytes; (i) BRIC 125 with cDE erythrocytes; (j) BRIC 125 with Endo F-treated cDE erythrocytes; (k) human monoclonal anti-D from cDE erythrocytes; (l) BRIC 125 immune complexes solubilized and digested with Endo F (see the Materials and methods section); (m) BRIC 125 immune complexes solubilized and incubated at 37 °C for 16 h [control for (l) above]. Tracks (a)–(d) are from the same experiment after 14 days autoradiography; tracks (e) and (f) are from the same experiment after 28 days autoradiography; non-specific precipitation of high- M_r material was apparent after prolonged autoradiography. (k)–(m) were from the same experiment after 21 days autoradiography. (g)–(j) were from the same experiment after 14 days autoradiography.

bands of M_r 47 000–52 000 and 100 000–120 000 (Fig. 1c). The band of higher M_r may be a dimer of the M_r 47 000–52 000 band. Parallel experiments using radioiodinated Rh_{null} cells did not give any strongly labelled bands in the immunoprecipitation (Fig. 1d). BRIC 125 clearly reacts with components that are different from the D polypeptide (Fig. 1a), and the R6A polypeptide (Ridgwell *et al.*, 1983). Although the BRIC 125 binding component could be detected in immunoprecipitates of Triton skeletons (Mueller & Morrison, 1981) from normal erythrocytes (Fig. 1e), the amount of labelling recovered in these immunoprecipitates suggests that the polypeptide is not quantitatively retained.

The diffuse nature of the M_r 47 000–53 000 band suggested that it might be glycosylated and so immunoprecipitation was carried out on normal erythrocytes which had been treated with a preparation containing endoglycosidase F and peptidyl-N-glycosidase F (Endo F preparation). Under these conditions the BRIC 125 reactive component formed a series of products of decreased M_r ranging from 29 000 to 42 000 (Fig. 1j). At least three different bands could be distinguished within this region, and these may represent intermediate forms carrying different numbers of N-glycans. The lowest- M_r product had an M_r similar to that of the D polypeptide (approx. 30 000) (Figs. 1g and 1h). The band of M_r 100 000–120 000 gave a corresponding series of products of lower M_r (Fig. 1j; range 75 000–100 000) consistent with these being dimers of glycosylated intermediates. In order to effect more extensive deglycosylation of the

BRIC 125-reactive components, the immunoprecipitate obtained from radioiodinated untreated normal erythrocytes was solubilized and treated with the Endo F preparation. Digestion with the Endo F preparation alone or in combination with endo- β -galactosidase under these conditions reduced the M_r 47 000–52 000 band to a single band of M_r 28 000 (Fig. 1l), which was clearly of lower M_r than the Rh(D) polypeptide (Fig. 1k). Treatment with endo- β -galactosidase alone had no effect on the mobility of the M_r 47 000–52 000 component (results not shown).

These results show that the BRIC 125 reactive protein is substantially glycosylated and the N-glycans do not appear to contain polylactosaminyl units since they are not susceptible to digestion by endo- β -galactosidase. The results suggest that BRIC 125 does not recognize an N-glycan-dependent epitope, since the smallest deglycosylated product can be immunoprecipitated from Endo F-treated red cells (Fig. 1j). The reactivity with BRIC 125 is also unaffected by sialidase treatment of intact red cells (Table 1). It is possible that the antibody recognizes an epitope on the polypeptide chain.

BRIC 125 does not react with either the LW or Fy blood group active glycoproteins

Although BRIC 125 is clearly not directed at the LW^{ab} antigen itself, since it reacts with erythrocytes lacking antigens of the LW blood group system [LW(a–b–); Table 1], the possibility existed that it recognized an epitope on the polypeptide(s) which carry

Fig. 2. Immunoblotting experiment using a murine monoclonal anti-LW^{ab} (BS56)

Tracks show developed immunoblots and autoradiographs of immunoprecipitates and erythrocyte membranes with or without treatment with Endo F. (a) Immunoblot using BS56 on cDE erythrocyte membranes; (b) immunoblot using BS56 on Endo F-treated cDE erythrocyte membranes; (c) autoradiograph of BRIC 125 immunoprecipitate from radioiodinated cDE erythrocytes after transfer to nitrocellulose; (d) autoradiograph of BRIC 125 immunoprecipitates from radioiodinated Endo F-treated cDE erythrocytes after transfer to nitrocellulose; (e) immunoblot of track (c) with BS56; (f) immunoblot of track (d) with BS56. High- M_r material present in immunoblots [tracks (e) and (f)] is, most probably, due to murine IgG (i.e. BRIC 125) from the immunoprecipitates.

the LW antigens. Immunoblots of BRIC 125 immunoprecipitates from normal cells were probed with monoclonal anti-LW^{ab} but did not reveal any reactive bands under conditions where blots of normal erythrocyte membranes gave a readily detected band corresponding to the LW^{ab} reactive component (Fig. 2c). The M_r of the LW^{ab} band was, as expected, significantly lower than that of the BRIC 125 polypeptide (Figs. 2a–2c). These results suggest that BRIC 125 does not react with the polypeptide(s) that carry the LW antigens.

The antigens (Fy^a and Fy^b) of the Duffy blood group system are located on a heavily N-glycosylated glycoprotein of similar M_r to that recognized by BRIC 125 (Moore *et al.*, 1982; Tanner *et al.*, 1987). In addition, certain human antibodies (denoted anti-Fy^s) have been described that react only weakly with Rh_{null} erythrocytes and not at all with Fy(a–b–) erythrocytes from Negroid donors (Race & Sanger, 1975). Since BRIC 125 reacts weakly with Rh_{null} erythrocytes, it was important to establish whether this antibody reacted with the Duffy glycoprotein. BRIC 125 gave normal agglutination reactions with Fy(a–b–) erythrocytes from Negroid donors (Table 1) and, furthermore, BRIC 125 immunoprecipitates did not react with human anti-Fy^a when examined by immunoblotting (results not shown).

It is clear that BRIC 125 identifies a previously uncharacterized polypeptide that is different from the D polypeptide, the R6A polypeptide and the component(s) that carry the LW antigens. Unlike these other com-

ponents, the BRIC 125 polypeptide is not entirely absent from the membranes of regulator type of Rh_{null} erythrocytes studied here.

BRIC 69 reactive components in normal erythrocytes

Another murine monoclonal antibody (BRIC 69) gave normal reactions with LW(a–b–) erythrocytes and Fy(a–b–) erythrocytes, but completely failed to react with Rh_{null} erythrocytes and thus differed in specificity from BRIC 125 (Table 1). The reactivity of BRIC 69 is similar to that previously described for monoclonal antibody R6A (Anstee & Edwards, 1982; Ridgwell *et al.*, 1983). Immunoprecipitation from radioiodinated erythrocytes using BRIC 69 gave a band of M_r 31000, together with a very diffuse staining band of M_r 35000–52000 (Fig. 3b). The band of lower M_r was the same as that obtained using R6A (Ridgwell *et al.*, 1983; Fig. 3c), although the band obtained with BRIC 69 contained more radioactivity. Inspection of the autoradiographs of the R6A immunoprecipitate also revealed a diffuse band of higher M_r similar to that found with BRIC 69. We assume that the increased radioactivity obtained in the BRIC 69 immunoprecipitate results from more efficient immunoprecipitation by this antibody. Treatment of BRIC 69 immunoprecipitates with Endo F resulted in the loss of both the diffuse band of M_r 35000–52000 and the band of M_r 31000 and gave rise to a new band which had an M_r similar to that of the D polypeptide (Figs. 3e and 3f), but which was quite distinct from that of the deglycosylated form of the BRIC 125 polypeptide (Fig. 3g). It is possible that one or other of the components present in the BRIC 69 immunoprecipitates results from coprecipitation rather than specific binding of BRIC 69 and that the deglycosylated forms of the two components coincidentally have the same M_r . Alternatively, both the different BRIC 69 reactive components in normal erythrocytes could be glycosylated forms of a common polypeptide and the differences in M_r of the reactive components would then be due to the presence of different degrees of N-glycosylation. Although the deglycosylated R6A polypeptide(s) has a similar M_r to that of the D polypeptide, we do not know if these proteins have related amino acid sequence.

Since the D polypeptide, the LW active component(s)

Table 2. Reactivity of BRIC 69 and BRIC 125 with blood cells and cell lines by immunofluorescence

+, More than 90% of cells strongly fluorescent; w, most cells very weakly fluorescent; neg, background fluorescence.

Cell	BRIC 69	BRIC 125
Mononuclear cells	neg	+
Granulocytes	neg	+
Platelets	neg	+
MOLT 4	neg	+
Raji	neg	+
Daudi	neg	+
IM9	neg	+
HL-60	neg	+
K562	w	+
HEL	w	+

Fig. 3. BRIC 69: autoradiographs of immunoprecipitates from radioiodinated intact erythrocytes and membrane skeletons and of Endo F-treated immunoprecipitates

All tracks show autoradiographs of immunoprecipitates using the following antibodies with different phenotypes of radioiodinated erythrocytes. (a) BRIC 69 with Rh_{null} erythrocytes; (b) BRIC 69 with cDE erythrocytes; (c) R6A with cDE erythrocytes; (d) human monoclonal anti-D with cDE erythrocytes; (e) BRIC 69 immune complexes solubilized and digested with Endo F (see the Materials and methods section); (f) BRIC 69 immune complexes incubated at 37 °C for 16 h [control for (e) above]; (g) BRIC 125 immune complexes solubilized and digested with Endo F; (h) BRIC 69 with membrane skeletons from cDE erythrocytes; (i) BRIC 69 with cDE erythrocytes. Tracks (a)–(c) are from the same experiment after 57 days autoradiography; (d)–(g) are from the same experiment after 34 days autoradiography; (h) and (i) were from the same experiment after 28 days autoradiography; non-specific precipitation of high- M_r material was observed.

Fig. 4. Localization of BRIC 126 antigen to distal renal tubular epithelial cell cytoplasm and to peritubular capillary endothelial cells (arrow)

Immunoperoxidase with haematoxylin counter-stain; $\times 975$.

and the BRIC 125 polypeptide are associated with the red cell skeleton, immunoprecipitation with BRIC 69 was carried out on skeletons isolated from radioiodinated normal red cells. The results show that both BRIC 69 binding components were present at low levels in the

skeletons and the intensity of the labelled bands suggested that these components were not quantitatively recovered in the skeletons (Fig. 3h). All of the components which are known to be absent from or deficient in Rh_{null} cells are associated, to a greater or lesser extent, with the red

Fig. 5. Immunoperoxidase staining of the human liver during development

(a) and (b), first trimester liver stained with R6A and BRIC 125 respectively; $\times 65$. Both antibodies stain haemopoietic cells and megakaryocytes, but BRIC 125 also reacts with other liver cells including hepatocytes. Note that megakaryocytes (arrows) show much more intense reactivity with BRIC 125. These differences are also apparent in sections of mid-trimester liver stained with R6A (c) and BRIC 125 (d); $\times 65$. Both antibodies react with material within the vessels of developing portal tracts (arrows). In the adult liver, R6A shows weak reactivity in sinusoidal spaces only (e), $\times 26$, but on a serial section, BRIC 125 also stains hepatocytes and sinusoidal cells (f); $\times 26$. In addition, this antibody shows extensive staining within the portal tract and bile ducts (arrow) are positive. Staining was carried out exactly as described in Sunderland *et al.* (1984).

cell skeleton. The absence or deficiency of these components from Rh_{null} cells suggests that they may form part of a functional unit in the normal red cell. It may be that a single genetic defect affecting the expression of one of these components causes the defective insertion of the other components of the complex into the membrane. Since Rh_{null} erythrocytes are known to have abnormal cell shape and cation permeability, it is possible that such a complex could be associated with the erythrocyte skeleton and be involved in a membrane transport process.

Reactivity of the antibodies with peripheral blood leucocytes, platelets and haemopoietic cell lines

The reactivity of BRIC 125 with peripheral blood cells was not confined to erythrocytes (Table 2). This broad pattern of reactivity was also observed with cultured cells (Table 2). This is in marked contrast to the reactivity of BRIC 69 which was specific for erythrocytes (Table 2).

BRIC 69 reacted weakly, if at all, with the erythro-leukaemic cell lines K562 and HEL (Table 2). In view of the broad distribution of BRIC 125 binding, it was of interest to determine if its weak reactivity with Rh_{null} cells was also reflected in its reactivity with lymphocytes from the same Rh_{null} donors. BRIC 125 gave similar reactions with lymphoblastoid cell lines from normals and individuals of the Rh_{null} phenotype, suggesting that these cells have normal levels of the BRIC 125 component. It seems likely that one, or more, of the erythroid specific components (D polypeptide, R6A binding component, LW components) are required for the normal assembly in the red cell membrane of a complex which contains the Rh antigens. The occurrence of such a complex could account for the abnormally low expression of the BRIC 125 binding component in Rh_{null} red cells, since the absence of one of the components of the complex could result in reduced expression of all of them. Since those non-erythroid cells reactive with BRIC 125 do not

contain either the R6A or BRIC 69 epitopes, the assembly of an analogous complex containing the BRIC 125 polypeptide in these cells may require the presence of other proteins related to those in the erythroid Rh complex.

Reactivity of BRIC 125, BRIC 69 and R6A with human kidney

Frozen sections of seven cadaveric kidneys were studied. Neither BRIC 69 nor R6A reacted with kidney tissue although R6A showed staining with a few scattered interstitial and intraglomerular cells in three of seven kidneys. In contrast, BRIC 125 and BRIC 126 bound to the endothelium of peritubular capillaries, arteries and veins, to arterial smooth muscle, and to the epithelial cells of the distal tubules (data for BRIC 126 shown in Fig. 4). The antibodies also gave strong diffuse staining of all glomerular structures and proximal tubules were negative. Human monoclonal anti-Rh(D) antibodies failed to react with kidney tissue (P. R. Evans, E. Hodges, E. M. Garvey, S. Shires, S. Nayyar, A. G. Maciver, M. Slapak & J. L. Smith, unpublished work).

Reactivity of BRIC 125, BRIC 69 and R6A with foetal and adult liver

The liver is a major site of haemopoiesis in the developing mammal. Frozen sections derived from six specimens each of first trimester and second trimester foetal liver, and from two specimens of adult cadaveric liver, were examined. R6A stained nests of haemopoietic cells in foetal livers from the first trimester (up to 12 weeks gestation) but other liver cell populations were unreactive (Fig. 5a). BRIC 69, in addition, showed a weak and diffuse reactivity with foetal hepatocytes. BRIC 125 gave a more extensive reactivity pattern and, in addition to haemopoietic cells, stained foetal hepatocytes, early sinusoidal cells and cells lining blood spaces. Megakaryocytes were very intensely stained (Fig. 5b). A similar reactivity was displayed in second trimester liver (12–24 weeks). By this stage, developing portal tracts with associated bile ductular epithelium were evident and, while these structures were negative for R6A and BRIC 69 (Fig. 5c), they were clearly reactive with BRIC 125 (Fig. 5d). Dense aggregates of antibody-reactive material which may represent enucleated red cells were present within some vessel lumens. In the adult liver, R6A reactivity was weak and confined to sinusoidal areas (Fig. 5e), while BRIC 69 again showed a weak and diffuse reactivity throughout the liver. BRIC 125 showed extensive reactivity in the adult liver, however, and stained sinusoidal cells, hepatocytes and portal tracts with their associated structures (Fig. 5f).

Concluding remarks

It is clear from these results that, although the two groups of antibodies recognize polypeptides that appear to be related because of their deficiency in Rh_{null} cells, they nevertheless identify epitopes with a quite different tissue distribution. BRIC 125 binds to a wide variety of cells and tissues, including peripheral blood cells and most of the cellular structures on human kidney and human foetal and adult liver. We have not identified the components reactive with BRIC 125 in non-erythroid tissues; however, if this antibody does indeed recognize

polypeptides that are related to those in the erythrocyte, then it seems likely that it has a function which is of general importance to the homeostasis of the cell. In view of the reported abnormalities in cation permeability found in Rh_{null} cells (Ballas *et al.*, 1984), it is possible that this polypeptide may have a role in the regulation of cation fluxes across the plasma membrane of mammalian cells.

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