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The apical and basal plasma membranes of the human placental syncytiotrophoblast contain different erythrocyte membrane protein isoforms. Evidence for placental forms of band 3 and spectrin *

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Using immunochemical techniques, we identified forms of erythrocyte membrane proteins in apical and basal plasma membranes of human placental trophoblast. A wheat germ agglutinin-binding intrinsic protein was present in the microvillous (maternal facing) but not the basal (fetal facing) membrane of the syncytiotrophoblast epithelium. Conversely, erythrocyte-related proteins of the basal membrane included two intrinsic membrane proteins, a 95000 M_r band 3 isoform and a form of spectrin. These four proteins were all absent from the microvillous membrane. The basal membrane spectrin isoform was also present in basal membrane skeletons. A 70000 M_r polypeptide which reacted with antibodies to band 3 was present in both microvillous and basal plasma membranes. Therefore, certain isoforms of red cell membrane proteins are polarized between the two surfaces of the human placental syncytiotrophoblast. We propose that the localization of spectrin to the basal membrane is related to the less bundled organization of microfilaments at this membrane compared with that of the microvillous membrane. The band 3 isoforms are candidates for participation in maternofetal anion transport.

Introduction

The human placental syncytiotrophoblast is a polarized epithelium located between maternal and fetal circulations. Consequently this cell layer plays the major role in maternofetal physiological interactions. The polarized structure of the syncytiotrophoblast is in many respects similar to that of other epithelial such as those of the intestine and kidney. Thus the syncytiotrophoblast is a unique model for the study of human epithelial cell structure and function. Many syncytial functions are mediated through one or the other

* This investigation was completed in collaboration with Dr. Martin Morrison shortly before his recent death.

Abbreviations: DMMA, dimethyl maleic anhydride; ConA, concanavalin A; WGA, wheat germ agglutinin; SDS, sodium dodecyl sulfate; PBS, 150 mM NaCl, 10 mM sodium phosphate (pH 7.4); ME, microvillous membrane erythrocyte-related protein; BE, basal membrane erythrocyte-related protein.

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of its two plasma membranes; an apical microvillous membrane that contacts maternal blood and a basal membrane that faces the fetal circulation [1]. It is therefore important to elucidate the composition of these two membranes to further our understanding of syncytial and indeed of epithelial cell structure and function.

The microvillous membrane is known to possess distinctive metabolite transport systems [2], receptor proteins [4,5] and structural proteins (Refs. 6–8, see Ref. 5 for review). In contrast, the basal membrane is less well characterized but is known to contain a glucose transporter [3] and transferrin receptor similar to those of the microvillous membrane [9,10], and a hormone sensitive adenyl cyclase [13].

Although certain common and distinctive proteins of the microvillous and basal membrane have also been investigated [12], knowledge of differences and similarities in the protein compositions of these two important membranes is still limited. On the basis of previous observations that antibodies raised to microvillous membrane cross-react with human erythrocytes (Booth, A.G. and Vanderpuye, O.A., unpublished observations, and Ref. 11) we have used antibodies to human erythrocyte membrane in immunochemical investigation of the two syncytial membranes. We report that the apical and basal plasma membranes of the syncytiotrophoblast contain different erythrocyte-related membrane proteins and discuss this finding in relation to the structural and functional differences between the two membranes.

Methods and Materials

Sources of proteins and antibodies

Human erythrocyte spectrin was from Calbiochem-Behring Corp, La Jolla, CA, U.S.A. Antibodies to band 3, were those previously described [24]. In brief, intact fresh red cells were digested with pronase to yield the 60000 M_r transmembrane fragment of band 3. Membranes were prepared [15], stripped of extrinsic proteins using 0.1 M NaOH extraction, washed and the proteins resolved on SDS gels. The region containing the 60000 M_r band 3 fragment was excised and used for immunization. The band 3 region of a nitrocellulose blot was cut out and used to affinity purify anti-band 3. Rabbit antibodies to human erythrocyte spectrin were obtained from Miles Scientific (Naperville, IL, U.S.A.). Production of rabbit antibodies to human glycophorins was by a method similar to that used for preparing anti-band 3. Briefly, crude glycophorins were prepared by the method of Hamaguchi and Cleve [47] and resolved by SDS gel electrophoresis [19]. A strip of gel was cut out and stained according to Zacharius et al. [48]. The band containing the glycophorins A, C and $(B)_2$ was cut out, washed, homogenized with 50 mM Tris-HCl (pH 7.8) and Complete Freunds adjuvant and injected into a rabbit.

Rabbit antibodies to human albumin, placental extract and erythrocyte membrane were obtained from Dako Corporation, Santa Barbara, CA, U.S.A. Agarose and reagents and apparatus for immunoblotting and immunoelectrophoresis were from Bio-Rad, Richmond, CA, U.S.A. Other antibodies and all other reagents were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Membrane preparation and fractionation

Syncytiotrophoblast basal and microvillous plasma membranes and erythrocyte membranes (red cell ghosts) were prepared as previously described [13–15]. In later preparations, a cocktail of protease inhibitors was included (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 10 μM leupeptin, 1 mM aprotinin, 10 μM chymostatin, 10 μM pepstatin). Membrane-associated cytoskeleton were prepared as described previously [7,8]. Extrinsic proteins were extracted with DMMA [6].

Immunochemical and analytical techniques

Immunoblotting was carried out as described [24]. Membrane proteins were solubilized for immunoelectrophoresis by the addition of Triton X-100 to 2% (v/v) to membranes (2–5 mg/ml of protein) followed by centrifugation at 30000 $\times g$ for 15 min and retention of the supernatant. Crossed immunoelectrophoresis and tandem crossed immunoelectrophoresis were performed as in Ref. 16. Fused line immunoelectrophoresis and crossed line immunoelectrophoresis and staining of gels for acetylcholinesterase followed previously described procedures [17,18,22]. Polyacrylamide

gel electrophoresis in the presence of SDS was performed by the method of Laemmli [19]. Protein and acetylcholinesterase were measured spectrophotometrically [20,21].

Immunoprecipitation of membrane vesicles using rabbit antibodies of various specificities was as follows: antibodies to human erythrocyte membrane, albumin or placental extract (0–100 μl) were added to membranes (50 μg of protein) in a total volume of 1 ml of PBS. Incubations with antibodies to erythrocyte membrane or albumin contained human albumin (1 mg). After incubation for 18 h at 4°C the membranes were sedimented by centrifugation (40000 × g, 15 min) and washed three times with PBS. Goat antibodies to rabbit IgG (50 μl of 48.5 mg/ml) were added to the resuspended pellets which were then incubated for 18 h at 4°C. At this stage visible precipitation occurred in some incubations. The suspensions were then centrifuged (3000 × g, 5 min) and the supernatants and resuspended pellets assayed for alkaline phosphatase activity [29]. Incubations with antibodies to albumin and placental extract served as negative and positive controls respectively.

Results

Identification of erythrocyte-related proteins in microvillous and basal membranes

In the microvillous membrane, two major antigens A and ME_1 and two minor antigens were recognized by antibodies to erythrocyte membrane (Fig. 1A) is crossed immunoelectrophoresis. Antigen A was identified as serum albumin by immunoelectrophoresis using specific antibodies (data not shown). Basal membrane proteins recognized by antibodies to erythrocyte membrane included A (albumin) and three antigens designated BE_1, BE_2 and BE_3 (arrows, Fig. 1B) which were distinct from the microvillous antigen ME_1 in mobility and appearance. Crossed immunoelectrophoresis of erythrocyte membrane proteins consistently yielded three major immunoprecipitates and occasionally others less intensely stained (arrows, Fig. 1C). Albumin was only barely detectable in the erythrocyte membrane preparation using antibodies to albumin in immunoelectrophoresis.

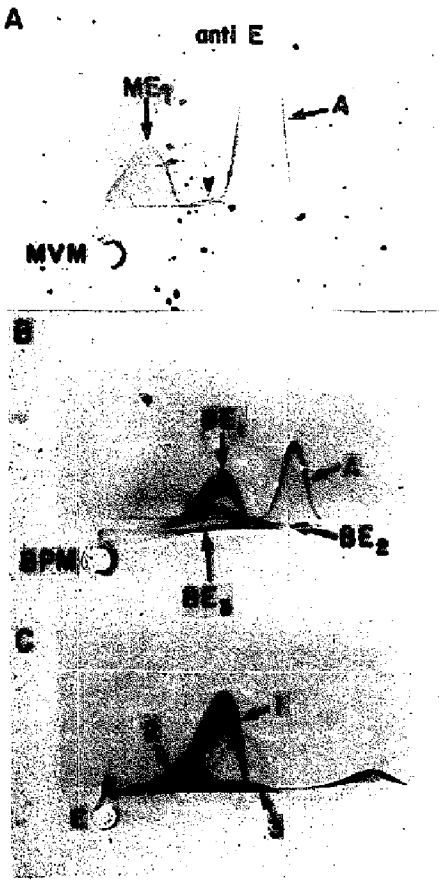


Fig 1. Crossed immunoelectrophoresis of microvillous (MVM) (1A), basal (BPM) (1B) and erythrocyte (E) (1C) membrane proteins against antibodies to erythrocyte membrane (anti E). Triton X-100 solubilized proteins from 50 μg of syncytial membrane proteins or 7 μg of erythrocyte membrane protein were applied to the sample wells. Electrophoresis in the first dimension was at 5 V/cm for 2 h (anode on right) and at 1 V/cm for 18 h in the second dimension (anode at top). The second dimension gel contained 5 μl/cm^2 of antibodies to erythrocyte membrane in these and subsequent experiments unless otherwise stated.

Erythrocyte contamination is not the source of the syncytial erythrocyte protein analogs

The specific activities of acetylcholinesterase a known erythrocyte intrinsic membrane protein [22], were 1.0 μmol/min per mg in erythrocyte membrane, 0.042 μmol/min per mg in basal membrane and 4 nmol/min per mg in microvillous membrane. Thus in the case of the microvillous membrane acetylcholinesterase activity was clearly negligible (250-fold less) compared with the erythrocyte membrane. In addition, crossed immunoelectrophoresis gels of the proteins of the three membranes using antibodies to erythrocyte membrane were stained for acetylcholinesterase activity. Basal and microvillous membrane protein loadings were 14-fold higher than those of erythrocyte to achieve similar immunoprecipitate sizes for basal and erythrocyte proteins. A single immunoprecipitate was stained in gels of erythrocyte membrane proteins but none were stained in gels of microvillous or basal membrane proteins (data not shown). However, the other trophoblast analogs were clearly observed. This result suggested that the trophoblast analogs did not arise from erythrocyte contamination.

Microvilli were immunoprecipitated with rabbit antibodies to the following antigens: erythrocyte membrane, albumin and placental extract. The microvillous membrane marker alkaline phosphatase [29,30] was measured in supernatants and pellets. (Fig. 2). Rabbit antibodies to erythrocyte membrane and placental extract but not anti-albumin efficiently mediated the precipitation of microvillous membrane vesicles with high efficiency. These results indicated that an erythrocyte membrane antigen was truly present on microvillous membrane.

Microvillous and basal membranes contain different erythrocyte membrane protein analogs

Since the preceding immunoelectrophoresis gels suggested that the two syncytial membranes contained different erythrocyte membrane proteins, the two membranes were compared by tandem crossed immunoelectrophoresis [16] using antibodies to erythrocyte membrane (Fig. 4). Because the two membrane preparations were known to contain serum proteins [12], anti-human serum was included in an intermediate gel to precipitate those

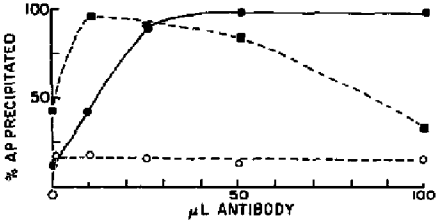


Fig. 2. Immunoprecipitation of microvillous membrane vesicles by antibodies to erythrocyte membrane, placental extract, or albumin. Membranes were incubated for 18 h at 4°C with 0–100 μl of antibody solution in a total volume of 1 ml of PBS. This included 1 mg of albumin in the case of incubations with anti-erythrocyte membrane and anti-albumin. Vesicles were washed three times with PBS and incubated for 18 h at 4°C with 50 μl of goat antibodies to rabbit IgG. Vesicles that had bound antibody and aggregated were sedimented at 3000 × g, 5 min. Alkaline phosphatase activities were measured in supernatants and resuspended pellets. Incubations were: filled circles, anti-erythrocyte membrane; filled squares, anti-placental extract; open circles, anti-albumin.

proteins (arrowheads, Figs. 4A–4C). Thus, the major syncytial erythrocyte protein analogs were not serum proteins. The microvillous-erythrocyte

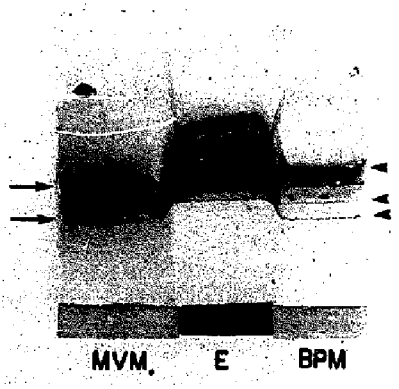
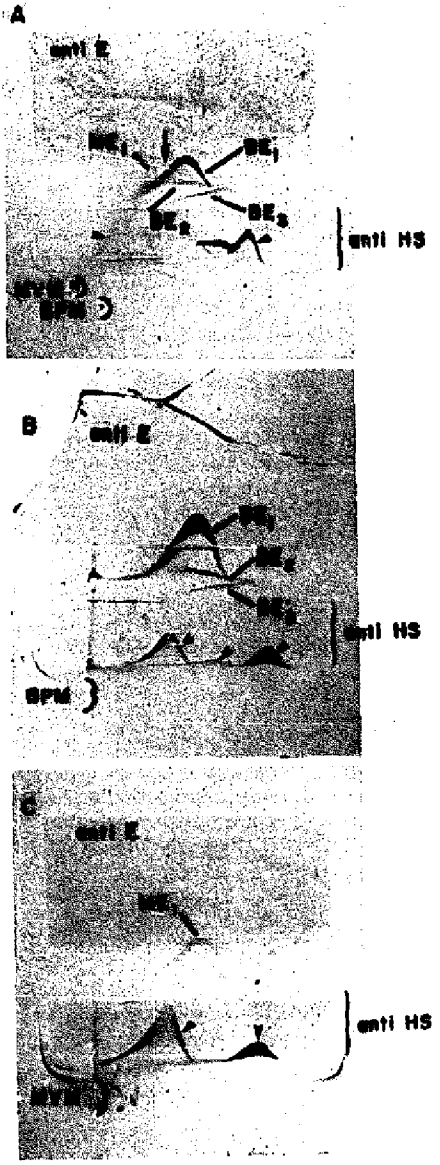


Fig. 3. Fused line immunoelectrophoresis of basal (BPM), erythrocyte (E) and microvillous (MVM) membrane proteins. Electrophoresis was into agarose containing antibodies to erythrocyte membrane (3 μl/cm²). BPM (300 μg protein), MVM (250 μg protein) and E (30 μg protein) in 300 μl of molten agarose were used to form troughs. Anode is at top, arrows indicate microvillous membrane-erythrocyte antigens and arrowheads indicate basal membrane erythrocyte antigens.



antigen immunoprecipitate (ME_1) did not fuse with any of the basal-erythrocyte antigen immunoprecipitates (BE_1, BE_2 or BE_3) (arrow, Fig. 4A). This result indicated that ME_1 was indeed a distinct protein present in microvillous but not basal membrane. Likewise, the basal membrane antigens BE_1, BE_2 and BE_3 were absent from microvillous membrane.

Properties of microvillous erythrocyte-related protein ME_1

To investigate the nature of the association of the microvillous-erythrocyte antigen (ME_1) with the lipid bilayer, microvillous membranes were treated with DMMA to obtain extrinsic and intrinsic membrane protein fractions [26,27]. The extrinsic protein fraction contained albumin (arrowheads, Fig. 5A) but ME_1 was present entirely in the intrinsic protein fraction (arrow, Fig. 5A). Crossed hydrophobic interaction immunoelectrophoresis [28] also showed that ME_1 was an amphiphilic protein (data not shown). After neuraminidase digestion, (2.45 U/5 mg membrane protein per ml as previously described [12]), the ME_1 precipitate was absent and a new cathodically migrating antigen appeared in its place (arrowhead, Fig. 5B). This reversal of electrophoretic migration suggested that ME_1 was a heavily sialylated protein, the polypeptide chain of which carried a net positive charge.

The glycoprotein nature of ME_1 was additionally demonstrated by crossed lectin affinity immunoelectrophoresis [17]. In the presence of WGA-agarose, the ME_1 immunoprecipitate was absent, indicating that the antigen had bound to

Fig. 4. (A) Tandem crossed immunoelectrophoresis of basal (BPM) and microvillous (MVM). Membrane proteins were electrophoresed through an intermediate gel containing antibodies to human serum (anti HS, 2 μl/cm^2) and then into agarose containing antibodies to erythrocyte membrane. Arrow indicates reaction of non-identity. BE_1–BE_3 are basal antigens and ME_1 is a microvillous antigen. (B and C) Crossed immunoelectrophoresis of microvillous (C) and basal membrane proteins (B) was performed through antibodies to human serum and into antibodies to erythrocyte membrane proteins (arrowheads, serum proteins). The microvillous antigen ME_1 and the basal antigens BE_1, BE_2 and BE_3 are not serum proteins.

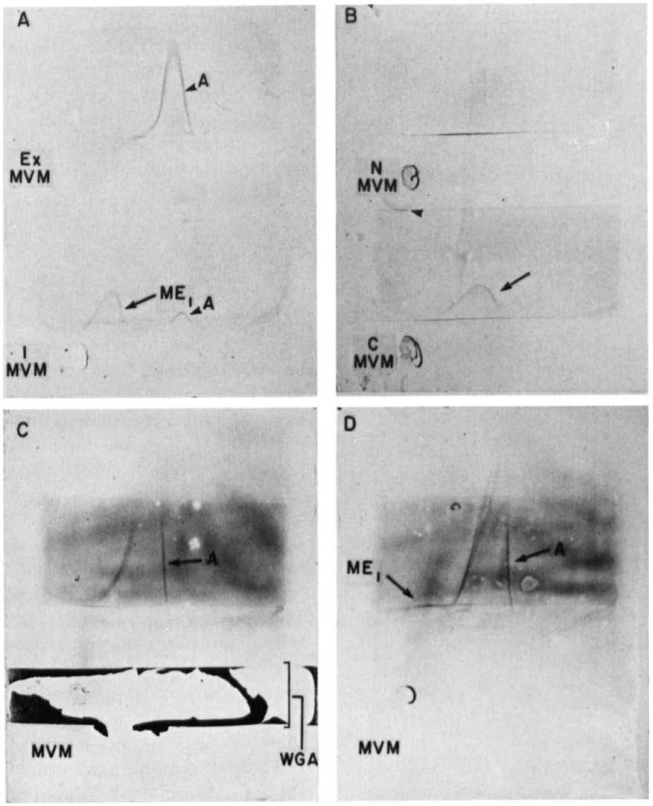


Fig. 5. (A) Crossed immunoelectrophoresis of microvillous extrinsic proteins (Ex) released by DMMA and intrinsic proteins (I). DMMA residue, against antibodies to erythrocyte membrane. Arrowheads, extrinsic proteins; arrows, intrinsic protein. (B) Crossed immunoelectrophoresis of microvillous membrane proteins treated with neuraminidase (N) or unexposed to enzyme (C) against antibodies to erythrocyte membrane. Arrow, ME_1 in control; arrowhead, ME_1 after neuraminidase treatment. (C and D) Crossed immunoelectrepitoresis of microvillous membrane proteins against antibodies to erythrocyte membrane with (C) or without (D) an intermediate gel containing WGA-agarose (WGA) (0–150 µl packed gel/ml agarose).

the matrix (compare Figs. 5C and 5D). The microvillous-erythrocyte antigen ME_1 was therefore identified as an intrinsic, WGA-binding sialoglycoprotein.

Properties of basal-erythrocyte related antigens, BE_1, BE_2 and BE_3

The membrane association and glycosylation of the basal erythrocyte related antigens were also investigated by the methods outlined above. Crossed immunoelectrophoresis of DMMA-produced fractions demonstrated albumin and antigen BE_3 in the extrinsic protein fraction (arrows, Fig. 6A) and BE_1 and BE_2 solely in the intrinsic protein fraction (arrowheads, Fig. 6A). Crossed hydrophobic interaction immunoelectrophoresis also revealed that BE_1, BE_2 and BE_3 bound to phenyl-Sepharose (data not shown) and were amphiphilic membrane proteins.

Antigens BE_1 and BE_2 were strikingly affected

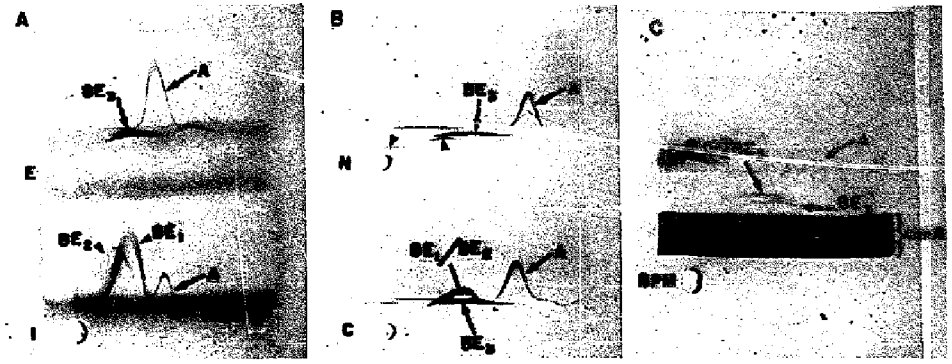


Fig. 6. (A) Crossed immunoelectrophoresis of basal membrane extrinsic proteins (E) and intrinsic proteins (I) fractions against antibodies to erythrocyte membrane. Arrow indicates extrinsic proteins; arrowheads, intrinsic proteins. (B) Crossed immunoelectrophoresis of basal membrane proteins treated with neuraminidase (N) or unexposed to the enzyme (C) using anti-erythrocyte membrane. Arrowheads, antigens affected by neuraminidase; arrows, antigens unaffected by neuraminidase. (C) Crossed immunoelectrophoresis of basal membrane proteins through an intermediate gel containing ConA-agarose (50 μl packed gel containing 0.79 mg ConA/ml agarose) and into a gel containing antibodies to erythrocyte membrane.

by neuraminidase; the sizes of their immunoprecipitates were much diminished and the mobility of one decreased (arrowheads, Fig. 6B). In contrast, albumin and BE_3 were unaffected (arrows, Fig. 6B). In the presence of ConA-agarose, the BE_1 and BE_2 immunoprecipitates were diminished while those of BE_3 and albumin were unaffected (compare Figs. 6C and 7C). These results, taken together, demonstrated that antigens BE_1 and BE_2 were glycosylated intrinsic membrane proteins and that BE_3 was an extrinsic protein.

Syncytiotrophoblast basal plasma membrane contains a spectrin-isoform

Basal and erythrocyte membrane proteins were subjected to tandem crossed immunoelectrophoresis against antibodies to erythrocyte membrane (Fig. 7B) and compared with a control gel in which only erythrocyte membrane proteins were analysed (Fig. 7A). Whilst fusion occurred between the three basal antigens and those of red cells, there was a 'spur' at the junction of the basal antigen BE_3 with erythrocyte antigen 3 (spectrin) (arrowhead, Fig. 7B). This spur suggested that the two proteins were antigenically related but not identical.

In addition, basal membrane proteins and spectrin were subjected to crossed line immunoelectrophoresis (as in Ref. 17). After electrophoresis through a zone of spectrin. BE_3 was absent whilst antigens BE_1 and BE_2 were unaffected (Fig. 7D) further demonstrating antigenic relatedness between BE_3 and spectrin. Fused line immunoelectrophoresis of basal membrane cytoskeletons, red cell membrane cytoskeletons and spectrin also showed that a spectrin-related protein was present in basal membrane but not in microvillous membrane (results not shown). On immunoblots red cell membrane spectrin subunits of M_r 240000 and 220000 were recognized by anti-spectrin (Fig. 8A, lane 1). In the basal plasma membrane sample, a polypeptide of M_r 240000 was recognized by antibodies to spectrin (Fig. 8A, lane 2). Because of the weaker recognition of β-spectrin by this antibody it was not possible to detect a trophoblast equivalent with this antibody. Microvillous membrane did not contain proteins similar to subunits of spectrin (Fig. 8A, lane 3). In addition, polypeptides of M_r 240000 and 220000 were efficiently precipitated in apparently equimolar amounts from either erythrocyte or basal membrane but not from microvillous membrane protein solutions. Finally, antibodies raised to basal membrane [12] reacted on immunoblots

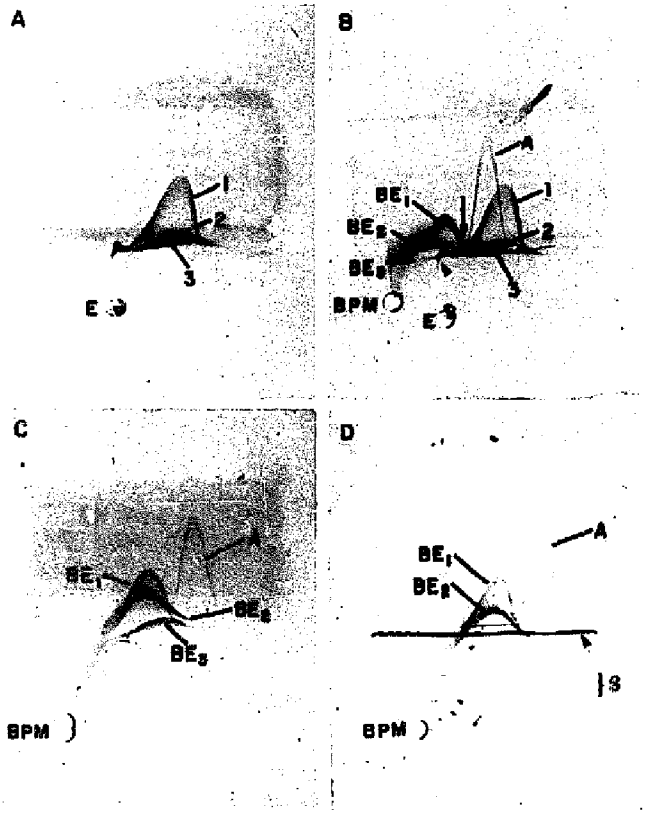


Fig. 7. (A) Crossed immunoelectrophoresis of erythrocyte membrane proteins (E) into antibodies to erythrocyte membrane as a control. (B) Tandem crossed immunoelectrophoresis of basal (BPM) and erythrocyte membrane proteins. The arrow indicates sites of fusion of basal and erythrocyte antigens immunoprecipitates. The arrow-head indicates a reaction of partial identity between basal antigen BE_3 and erythrocyte spectrin. (C) Crossed immunoelectrophoresis of basal membrane (BPM) proteins against antibodies to erythrocyte membrane as a control for Fig. 7D. (D) Crossed line immunoelectrophoresis of basal membrane proteins (40 μg) through a trough containing purified erythrocyte spectrin (S) (3 μg/ml agarose) and into antibodies to erythrocyte membrane.

with the α-subunit of purified spectrin (results not shown), considered together, these results indicated that (1) a spectrin analog was present in the basal but not the microvillous membrane of the syncytiotrophoblast and (2) suggested this basal membrane spectrin was distinct from erythrocyte spectrin.

Band 3 analogs in syncytiotrophoblast plasma membranes

Immunoblotting with affinity purified antibodies to band 3 revealed band 3 and previously described catabolic fragments of M_r 60000; 40000 and 20000–30000 [24] in a blot of red cell membrane proteins (Fig. 8B, lane 1). A polypeptide of M_r 95000 was recognized by anti-band 3 in basal

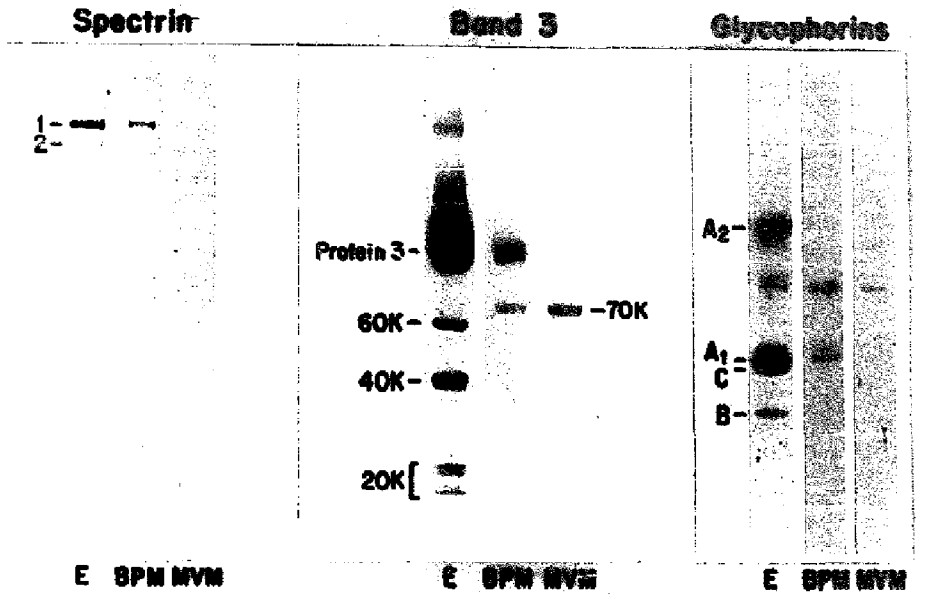


Fig. 8. (A) Immunoblotting of microvillous (MVM), basal (BPM) and red blood cell (E) membrane proteins using antibodies to human erythrocyte spectrin. Identical amounts of membrane proteins were subjected to electrophoretic transfer to nitrocellulose and reacted with anti-spectrin followed by ^{125}I-protein A. (B) Immunoblotting of microvillous, basal and red blood cell membranes with antibodies to human red blood cell band 3. Protein bands labeled 60K, 40K and 20K are metabolic fragments of red cell membrane band 3. (C) Immunoblotting of microvillous, basal and red blood cell membranes with antibodies to human red blood cell glycophorins A, B and C. Glycophorin A dimer (A_2), glycophorin A monomer (A), glycophorin B (B) glycophorin C (C).

(Fig. 8B lane 2) but not microvillous membrane proteins (Fig. 8B, lane 3). Both microvillous and basal membranes contained a polypeptide of M_r 70 000 cross-reactive with anti-band 3. Thus, one analog of band 3 was restricted to the basal membrane but another form of lower M_r, perhaps a fragment, was present in both microvillous and basal membranes. The latter species was clearly different in mobility than any species from red cell membrane and conversely the characteristic catabolic fragments of erythrocyte band 3 were never detected in basal or microvillous membranes.

Reactivity of antibodies to glycophorins with syncytiotrophoblast plasma membranes

Antibodies to glycophorins (A, B and C) were tested on blots of proteins of syncytiotrophoblast and erythrocyte membranes (Fig. 8C) A protein intermediate in M_r between glycophorin A dimer and monomer was present in all three membranes. Microvillous membrane clearly lacked proteins corresponding to glycophorins A, B. C. In the case of basal membranes, interpretation was more complicated. An immunoreactive doublet migrated at the position of glycophorin A monomer and could correspond to either analogs of glycophorin and C monomers or glycophorin A monomer and B dimer. Since basal membrane lacks bands corresponding to glycophorin A and C homodimers and heterodimers it is difficult to identify the crossreactive proteins with specific glycophorins. The basal membrane pattern is very distinct from that of erythrocyte membrane and this suggests that possible low level contamination by the latter contributes only very little to this profile.

Discussion

This study demonstrates that the apical and basal plasma membranes of the human placental syncytiotrophoblast contain different erythrocyte protein analogs. Although in the case of Bpm a low level of erythrocyte contamination seems possible, substantial evidence indicates that the occurrence of these analogs is not due to erythrocyte membrane contamination: (a) acetylcholinesterase activities and major glycophorins are low or absent in syncytial membranes, (b) antibodies to erythrocyte membrane precipitate microvillous membrane vesicles, (c) syncytial membranes contain only a limited subset of red cell membrane protein analogs with qualitative differences in spectrin, band 3 and other proteins from those of the red cell membrane. Our results therefore confirm and extend the finding by Johnson et al. [11] that three monoclonal antibodies raised against microvillous membrane cross-react with non-serum, non-blood group erythrocyte antigens.

Two considerations are of particular interest: (1) the distribution of the red cell protein analogs between the two plasma membranes of the syncytiotrophoblast, and (2) the possible functions of these proteins in the syncytiotrophoblast.

The identification of a basal plasma membrane form of spectrin extends our previous observation of high M_r proteins (> 200 000) in basal but not microvillous membranes. In various cell types, filamentous actin crosslinking, intermediate filament binding and membrane association properties have been described for non-erythroid spectrins [31–33,35–38].

Thus, in the syncytiotrophoblast, the distribution of spectrin may be related to the differing arrangements of actin or other cytoskeletal filaments at the two cell surfaces of this epithelium. At the apical membrane, microfilaments are cross-linked into relatively prominent bundles within microvilli oriented perpendicularly to the apical surface. In contrast, basal membrane-associated microfilaments are less conspicuously bundled and run parallel to the membrane [34]. Such differences must involve the effects of different actin-binding proteins. The spectrin isoform at the basal membrane could thus be functionally involved in the distinctive organization of actin filaments of that membrane. Interestingly, an analogous localization of spectrin seems to occur in intestinal and renal epithelial cells. In these cells types, spectrin is deficient or absent from microvilli and more abundant in the terminal web and basolateral membranes [43,44,40].

Two band 3-related polypeptides can be detected and these differ in their occurrence in the two syncytiotrophoblast plasma membranes. One isoform of band 3 of M_r 95 000 is restricted to basal membrane. In contrast, another isoform of M_r 70 000 was present in both membranes. The 70 000 M_r polypeptide could be either a proteolytic fragment or, alternatively, represent one of two trophoblast forms of band 3. We feel that the 95 000 M_r protein is not erythrocyte band 3 because its catabolic fragments were never seen in basal membranes. By analogy with red cell band 3, the syncytial 70 000 M_r protein may represent a membrane integrated polypeptide that lacks the glycosylated COOH terminal 25 000 M_r domain. Other workers report proteins cross-reactive with antibodies to band 3 which include species of M_r 60 000 [41]; 120 000 [40] and 68 000 [45]. The recent report of the cloning of a human non-erythroid band 3 gene and reports of DIDS-inhibitable anion transport in a number of cells [42], suggest that band 3 analogs may be of considerable physiological significance. Consistent with our findings are recent reports of DIDS-inhibitable anion exchange mechanisms in human placental microvillous membrane vesicles [39].

The glycosylated intrinsic membrane proteins BE_1 and BE_2 appear to be unique to the basal membrane and therefore may not correspond to band 3 isoforms. The basal membrane protein comigrating with glycophorin A probably represents one of these proteins. Conversely, the WGA-binding intrinsic protein ME_1 is restricted to the microvillous membrane and also remains to be identified with a known red cell membrane protein. These proteins are likely to mediate functions that are restricted to an individual plasma membrane. For example, placental and erythrocyte membranes have been reported to contain an immunologically similar folate receptor that is a glycosylated intrinsic protein [46], which could correspond to ME_1. In conclusion, we propose that the distribution of erythrocyte membrane

protein analogs in the syncytiotrophoblast reflects the overlap and polarization of specific functions at the syncytiotrophoblast cell surfaces as well as the differing organization of the apical and basal plasma membranes.

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