Red Cell Membrane Antigens Common to Several Mammalian Species[1] (36106)

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Differences in protein composition of red cell membranes of non-human primates and red cell membranes of several mammalian species were demonstrated by physicochemical methods (1, 2). Immunological studies of solubilized human red cell membrane proteins have detected 5–7 antigenic determinants associated with such protein components. The majority of these antigens were shown to be localized in deeper layers of the membrane (3). Studies on one of the membrane proteins (spectrin) isolated from human and three other mammalian red cell membranes have shown that they are immunologically related (4). This preliminary report describes our studies on the immunologic reactivity of red cell membrane proteins of several mammalian species with antisera prepared against human membrane proteins.

Materials and Methods. Erythrocytes. Human red blood cells were collected in acid–citrate–dextrose and stored no longer than two to three weeks prior to use. Sheep, ox, rat, pig, horse, cat, dog, and rabbit red cells were obained in Alsever's solution from Pentex Biochemicals (Miles Laboratories, Kankakee, IL). The cells were washed four times with isotonic saline before hemolysis.

Preparation of Stromal Proteins. Ghosts were prepared by osmotic lysis and phosphate buffer washes according to the method of Rega *et al.* (5) until complete removal of hemoglobin. Stromata were extracted in the cold with *n*-butanol at pH 2.1 (6). The waterphases thus obtained were dialyzed against distilled water and concentrated by pervaporation prior to gel filtration.

Solubilization of stromal proteins was also performed by dialysis of hemoglobin free ghosts against water alkalinized to pH 9.5 (7).

Chromatography. The waterphases were chromatographed on Bio-gel P-100 (Bio-Rad Laboratories Calbiochem, Los Angeles, CA) in formic acid (0.05 *M*) as previously described (4, 6).

Immunoelectrophoresis and immunodiffusion. The micro method of Scheidegger was performed in 1% agar in barbital, calcium lactate buffer (8). Ouchterlony immunodiffusion was done in agar prepared in the same buffer.

Preparation of antisera. New Zealand rabbits were immunized with peak B of human pH 2 waterphase (3). Fifty milligrams of antigen solubilized in 8 *M* urea were injected into the foot pads and back muscles over a period of six months with complete Freund's adjuvant. To make resulting antisera specific for erythrocyte membrane proteins, 1 ml of each respective antiserum was absorbed with 15 mg of lyophilized human serum and 10 mg of human globin. After incubation at 37° for one hour, the antisera were left overnight at 4° and then centrifuged. The procedure was usually repeated at least twice. Specificity of the antisera was checked by immunoelectrophoresis and Ouchterlony test with the respective plasma and hemolysates as obtained during preparation of the ghosts. Details regarding the specificity of the antiserum used (R-44) in these studies were reported elsewhere (3).

Results and Discussion. The pH 2 waterphases from red cell membranes of the seven species studied were fractionated on Bio-gel P-100 in 0.05 *M* formic acid. Elution patterns obtained with the mammalian mem-

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branes were similar to those described for human red cell membrane proteins (6). The membrane protein fractions (referred to as peaks A, B, and C) were recovered in about the same relative quantitative proportions, regardless of the species used. Figure 1 shows the elution profile of a dog pH 2 waterphase. Peak B materials of the different species were chosen for comparative immunological analysis. These fractions contained disaggregated membrane proteins which were readily soluble and thus more suitable for immunodiffusion and immunoelectrophoretic studies (3). Five percent solutions of fraction B of the different species were prepared in ammonium bicarbonate buffer (0.02 *M*, pH 8.3) and used for immunodiffusion and immunoelectrophoresis. The fraction of human origin was almost completely solubilized in this buffer. However, materials of peak B of non-human species showed different degrees of solubility. A typical immunoelectrophoretic pattern of human fraction B is shown in Fig. 2a. Five to seven immunologically different antigens can be identified. Sheep, ox, pig and horse red cell membrane B fractions

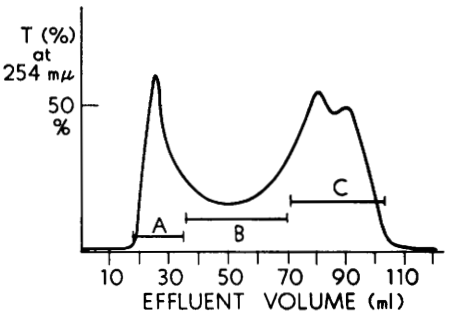

FIG. 1. Gel filtration pattern of dog pH 2 waterphase on Bio-gel P-100 column (35 cm × 2 cm). Elution with 0.05 *M* formic acid.

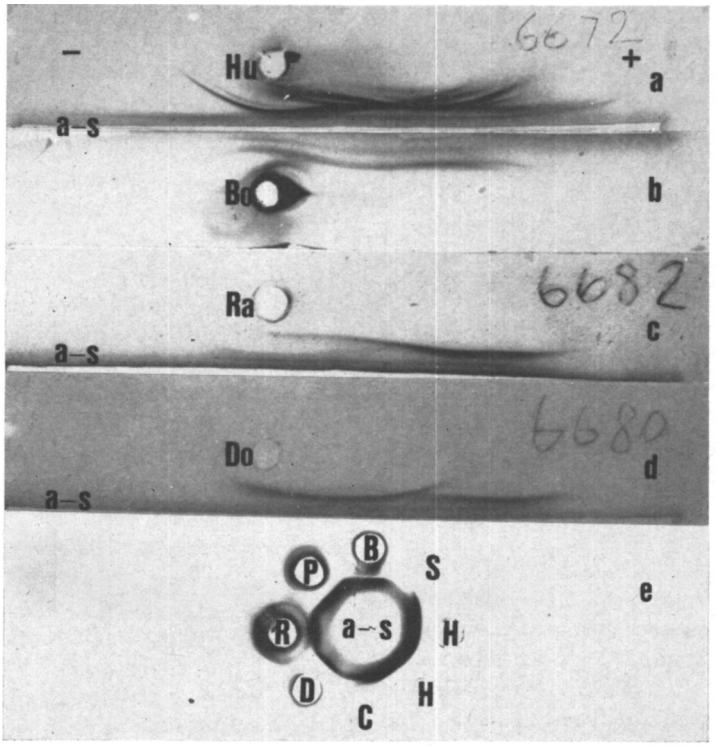

FIG. 2. Immunoelectrophoretic patterns obtained with gel filtration peak B of pH 2 waterphases: a. human peak B; b. bovine peak B; c. rabbit peak B; d. dog peak B; e. Ouchterlony double diffusion patterns of stromal proteins solubilized by alkalinized water (pH 9.5). Human (H), cat (C), dog (D), rabbit (R), pig (P), bovine (B), and sheep (S). a-s rabbit anti-human peak B antiserum (R-44).

afforded one major precipitin line in the position of α_2-β globulins. A typical pattern obtained with bovine peak B is shown in Fig. 2b. This material was difficult to dissolve (note the presence of a large amount of protein at the application site) and the presence of a long doubled precipitin is indicative of the poor solubility of this aggregated material. Gel filtration peaks B of cat, rabbit and dog, red cell membranes were more soluble and yielded a number of precipitin bands by immunoelectrophoresis. Two antigens were detected in cat and rabbit fraction B. The strongest precipitin line was found in α_2 globulin position and another fainter in β globulin position, in addition to a long precipitin line extending from α_2 to β globulin regions. Figure 2c demonstrates such a pattern for the rabbit material. Dog stromal protein preparations gave at least two heavy precipitin lines; one in the α_2-β position, and one in the α_2-α_1 position. Again, a third long precipitin line was found, extending from the α_2 to the β regions (Fig. 2d).

Comparative immunodiffusion studies of the solubilized membrane proteins of the eight species obtained by dialysis against water (pH 9.5) are shown in Fig. 2e. One major precipitin line was obtained with each of the membrane preparations. A high degree of immunological similarity of this antigen among the species is demonstrated by the confluence of the precipitin lines. The only exception is the human antigen which shows the presence of one additional antigenic determinant by formation of a spur.

Immunologic relationships were also demonstrated by absorption of the antisera with the different membrane protein preparations. When antiserum R-44 was absorbed with dog peak B protein (10 mg/ml), and used in immunoelectrophoresis of rabbit or dog peak B, no precipitin lines formed under these experimental conditions. This, also, markedly decreased the precipitin line formation quantitatively and qualitatively with human peak B material. Second absorption of the antiserum with the same amount of the antigen completely removed the antibodies reacting with the human material. When human peak B was used for similar absorption experiments, 2 mg/ml were sufficient to remove all precipitating antibodies. In spite of the presence of high titer of cross-reacting precipitating antibodies in the sera of the immunized animals, damage to their red cells could not be demonstrated. There was no overt or occult hemolysis detectable. This finding would indicate that the antigens are not available on the surface of the cell and are probably located in the deeper layers of the membrane (3).

The results suggest that the structural proteins of the red cell membranes of different species may have similar chemical structures. This notion is supported by our findings that antisera prepared against human stromal proteins react with stromal proteins of other species. One or more antigenic determinants of the various species are very closely related as was shown by immunodiffusion and immunoelectrophoresis. Our results also confirm the recent findings of Tillack *et al.* (4), who showed that spectrin prepared from red cell membranes of four animal species were immunologically related. The physicochemical characteristics of the four different spectrins were remarkably similar, and thus, further support the notion of structural similarities of at least parts of the protein structures. The poor solubilities of some of the preparations do not allow more meaningful comparative studies at the present time. Better methods will have to be developed to provide materials of high solubility for preparative and immunological work. Until then, one can only speculate whether the antigenic determinants demonstrated are carried on one or several membrane protein molecules.

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