Acta Dermatovener (Stockholm) 57: 493–496, 1977

PBS-SOLUBLE SUBSTANCES WITH BLOOD GROUP ACTIVITY FROM HUMAN EPIDERMIS AND DERMIS[1]

Luis A. Diaz, Nickolas J. Calvanico, Thomas B. Tomasi, Jr and Robert E. Jordon

From the Cutaneous Immunopathology Unit and the Departments of Dermatology and Immunology, Mayo Medical School and Foundation, Rochester, Minnesota, USA

Abstract. Phosphate-buffered saline (PBS) soluble extracts of human epidermis and dermis from secretor donors contain products with blood group antigenic activity. The predominant blood group found in a sample was the same as the red cell phenotype of the donor. H substance activity was present in all PBS-soluble products (from A, B and O donors). By gel filtration chromatography on a Bio-Gel A−1.5 m column, the epidermal and dermal PBS-soluble blood group substances fractionate in the void volume.

Key words: Blood group substance; Immunofluorescence; Hemagglutination; Chromatography

The intercellular substance (ICS) of human epidermis contains blood group substances, as demonstrated by mixed agglutination (2, 8, 19) and immunofluorescence (IF) techniques (5, 8, 14, 17). By contrast, the dermis contains blood group antigens in its capillaries and blood vessels, as well as in sebaceous and sweat glands (8, 17), as determined by IF technique. Homogenates of whole skin were found to absorb antibodies against blood group antigens A and B (13).

In the present study, homogenates of pure epidermal and dermal tissues were prepared in phosphate-buffered saline (PBS) from A, B, and O secretor donors. The soluble products of both epidermis and dermis were chromatographed and then investigated for their content of blood group substances by inhibition of the hemagglutination (HA).

MATERIALS AND METHODS

Extraction procedures. Fresh normal human skin was obtained from surgical specimens. The blood type (ABO, Rh, Lewis A and Lewis B) and the secretor status of the patient donor were determined. The subcutaneous tissue and reticular dermis were first carefully separated from the skin with a scalpel. The skin specimens were then washed in distilled water at 4°C and treated with 2 N NaSCN at 4°C for 3 to 5 hours. Treatment of the skin with 2 N NaSCN produces a clean separation of the epidermis from the dermis (7). The clean epidermal and dermal sheets were minced and homogenized separately *ad modum* Fisher (3) in PBS at pH 7.2 (0.14 M NaCl, 0.1 M phosphate). After centrifugation the PBS-soluble products from the epidermis (Ex-1-E) and those of the dermis (Ex-1-D) were dialysed against distilled water and then lyophylized.

From approximately 30 g of separated epidermis, 1 g of Ex-1-E was recovered, while 30 g of dermis yielded about 4–5 of Ex-1-D.

Chromatography. The soluble products from the epidermis and dermis were fractionated on 2.5×110 cm column of Bio-Gel A 1.5 m (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with 1.0 M sodium chloride. Pools from representative peaks were dialysed against distilled water at 4°C overnight, lyophylized and tested for blood group substances and NHS components.

Detection of blood group substances by direct HA and inhibition. The HA titer of human anti-A, anti-B (Ortho Diagnostics, Raritan, N.J.) and a PBS solution of crude hemagglutinin 1 of *Ulex europeus* seeds (12) against human A, B, and ● erythrocytes was determined using a microtiter system. The end point titer is the equivalent of one HA unit. HA inhibition studies were performed ac-

Abbreviations

ICS	Intercellular substance
IF	Immunofluorescence
PBS	Phosphate-buffered saline
Ex-1-E	Extract of epidermis
Ex-1-D	Extract of dermis
HA	Hemagglutination
BMZ	Basement membrane zone
S-XIV	Type XIV pneumococcus polysaccharide
NHS	Normal human serum
FITC	Fluorescein isothiocyanate

[1] This work was presented in part at the American Federation for Clinical Research, Atlantic City, New Jersey, May 4, 1975.

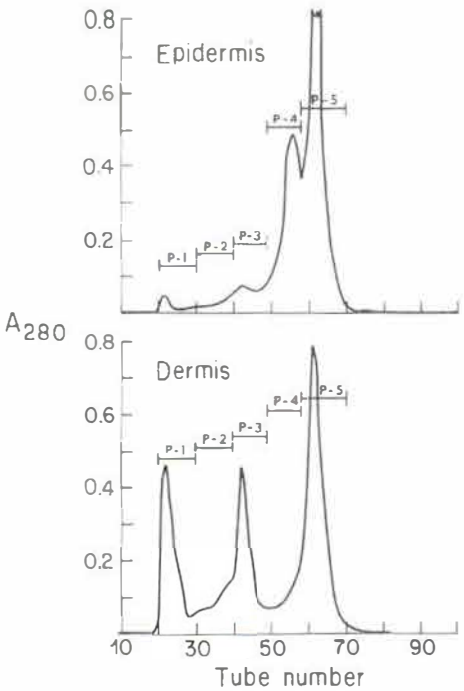

Fig. 1. Chromatogram of the epidermal and dermal extracts on Bio-Gel A-1.5 m (120×2.5 cm) in 1.0 M NaCl.

cording to Kabat (10). A dilution of antisera or lectin defined in HA units was incubated with doubling dilutions of the test fractions (epidermal, dermal and controls). The inhibition of the HA by the antigenic fractions was recorded at the end of 3 and 24 hours.

Immunodiffusion. Double immunodiffusion was carried out as described by Ouchterlony (15).

IF techniques. Horse anti-type S-XIV (615, 1938 bleeding) donated by Dr E. A. Kabat, was conjugated with fluorescein isothiocyanate (FITC) following the technique of Johnson & Holborow (9). The molar F/P ratio was 3.24. Cryostat sections of monkey esophagus and human skin were used as substrates. FITC-labeled horse anti-S-XIV serum absorbed in S-XIV was used as a control for specificity.

Results

Chromatography of the soluble products from epidermis and dermis. The elution patterns of the epidermal (Ex-1-E) and dermal (Ex-1-D) PBS-soluble material from the column of Bio-Gel A-1.5 m in 1.0 M NaCl are shown in Fig. 1. Tubes were pooled as shown (P-1, P-2, P-3, P-4, P-5).

Detection of NHS components and horse anti-S-XIV reactive substances. By Ouchterlony analysis with goat anti-normal serum (NHS), components of NHS were found in pools 2 and 3 of both epidermal and dermal chromatographic fractions. Very weak reactions were found in pools 1, 4, and 5 (Fig. 2). Neither the epidermal nor the dermal fraction reacted with horse anti-S-XIV serum when tested by double immunodiffusion at room temperature.

Detection of blood group substances by inhibition of the direct HA. Both the epidermis and dermis of blood group O secretor donors contained substances that produce inhibition of the HA of human O erythrocytes by hemagglutinin I of *Ulex europeus* (Table I). The same epidermal and dermal products produce very little inhibition of HA in the A-anti-A and B-anti-B systems, as seen in Table I. The O inhibitory products were found in the exclusion volume of the Bio-Gel A-1.5 m column (pool 1) when the epidermal and dermal extracts were chromatographed. In cases where the donor was a blood type A or B secretor, substances with A or B antigenic activity respectively were found in the same chromatographic fractions. These extracts or fractions also contain small amounts of H inhibitory products. The relative amounts of these inhibitory substances, in extracts of these donors, parallel those shown in Table I for an O secretor donor. The control antigens, ovalbumin, NHS, chymotrypsinogen, did not produce inhibition of the agglutination.

Immunofluorescence. The direct IF staining using fluorescein-labeled horse anti-S-XIV serum on monkey esophagus revealed a weak granular staining of the ICS. On human skin substrate (Fig. 3) the same conjugate produced a bright fluorescence of the whole epidermis. With both monkey esophagus and human skin, a weak linear staining of segments of the basement membrane zone (BMZ) (Fig. 3) and outline of the basal cells are present. Absorption of horse anti-S-XIV serum with S-XIV abolishes the staining.

DISCUSSION

In the present study we have demonstrated that both human epidermis and dermis from secretor A, B and O donors contain PBS-soluble substances with blood group activity as demonstrated by inhibition of the HA. By chromatography these products displayed a single peak in the exclusion volume of the Bio-Gel A-1.5 m column. Furthermore, they

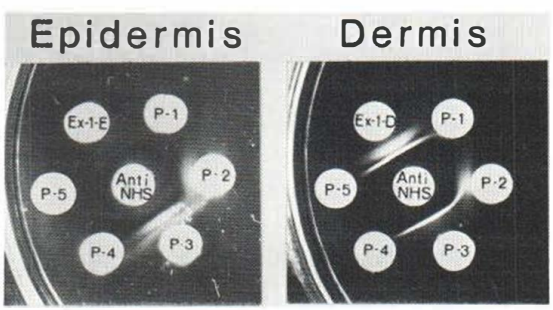

Fig. 2. Double immunodiffusion of epidermal and dermal fractions (peripheral cells) against goat anti-NHS (center well).

Table I. *Hemagglutination-inhibition*[a]

Skin donor: blood group O, Rh−, Let(A)−, Le(B)+, secretor

Antigens	*Ulex europeus*-O System	A-Anti-A System	B-Anti-B System
Ex-1-Epidermis	125	500	500
P-1	31.25	250	500
P-2	250	500	500
P-3	500	NI	NI
P-4	NI	NI	NI
P-5	NI	NI	NI
Ex-1-Dermis	31.25	100	100
P-1	4.1	25	50
P-2	62.5	50	100
P-3	500	NI	NI
P-4	NI	NI	NI
P-5	NI	NI	NI

[a] Amounts (in μg) of epidermal and dermal antigens which produce complete inhibition of the agglutination in the O-Anti-O System (4 HA units), A-Anti-A System (8 HA units), B-Anti-B System (8 HA units). NI: No inhibition, up to 500 μg.

contain very few NHS contaminants. The strongest blood group activity in the PBS-soluble products of epidermis and dermis seems to correlate with the red cell phenotype of the skin donor. The presence of a certain blood group activity different from that of the skin donor, may be a result of cross reactivity between these substances (1). The donors of skin in the present study were all secretors. Currently we are investigating the presence of blood group activity in the PBS-soluble products of skin from non-secretor individuals.

Blood group antigens [A, B, H, Le(A), Le(B)] in man exist in two forms (6, 11), one as a glycolipid present in red cell and other cell membranes which cannot be extracted with water or salt solutions, but which are dissociable from the red cell membrane by extraction with organic solvents. They constitute the so-called alcohol-soluble blood group antigens. A second population of high molecular weight compounds having blood group activity is found in body fluids and secretions. These compounds are water-soluble glycoproteins composed of carbohydrate and peptide moieties. Which type of blood group substance, either glycoprotein or glycolipid, is distributed in different tissues and cells is not well known (6). In fact, both blood group glycolipids and glycoproteins coexist in many organs and tissues (6). It is also known that the glycoprotein blood group antigens present in secretions or other cells are inherited as a Mendelian character (16). In individuals carrying the Se gene (homozygous and heterozygous), secretion of these glycoproteins occurs, whereas no secretion of these antigens occurs in those individuals homozygous for the allele Se. Some recent studies (4) have suggested that the glycolipid blood group antigens may be independent of the secretor genes.

Szulman (17) found in IF studies that stratified epithelia and sweat glands display blood group antigens in the walls of epithelial cells, irrespective of the secretor status of the donor. Furthermore, the staining seen with IF was unaffected by water treatment of the skin, though incubation of the skin in ethanol abolishes this specific staining (8, 17). The origin and relation of the PBS-soluble skin products with the known blood group antigens [A, B, H, Le(A), Le(B)] remain to be determined. Contamination of both tissues by red cell antigens released during the extraction procedure must be taken into consideration. However, we believe that these antigens are native to the epidermis and dermis because of the relatively constant amounts of PBS-soluble antigens extracted from the tissues of these secretor donors. In the case of the dermis, sweat and sebaceous glands, blood vessels, and hair follicles (8, 17) may have contributed to some extent, but in addition, the ground substance may also be an important source of the PBS-soluble products having blood group activity, as described here. It was mentioned previously that human skin contains blood group substances A, B and H. The possibility that this tissue may also contain precursor molecules for these blood group antigens was investigated by direct IF using a FITC-labeled

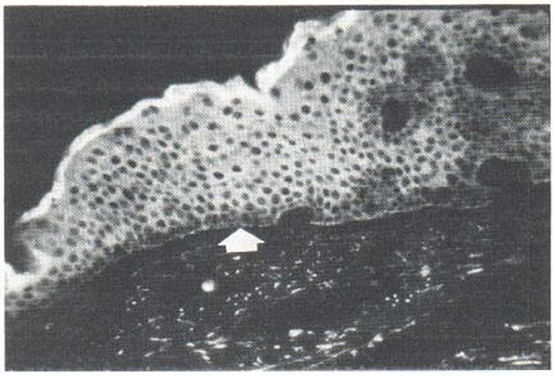

Fig. 3. Direct IF staining with horse anti-S-XIV serum (molar F/P=3.24) on normal human skin substrate. Arrow indicates BMZ staining.

horse anti-S-XIV serum. It is known that horse anti-S-XIV serum cross reacts with precursor molecules of blood group substances (6, 11, 18). The direct IF studies described here, using fluorescein-labeled horse anti-S-XIV, demonstrate a staining of the skin ICS and BMZ having an unusual granular pattern, which is in agreement with the general distribution of blood group antigens in the ICS of the epidermis. The absence of reactivity in the epidermal and dermal fractions with horse anti-S-XIV when tested by immunodiffusion is not understood at present but may be explained by several factors that affect the sensitivity of this technique. This phenomenon is presently under investigation.

ACKNOWLEDGEMENTS

This work has been supported in part by research grants AI12049 and AM 17554 from the National Institutes of Health, Public Health Service and by a grant from the Minnesota Chapter of the Arthritis Foundation.

REFERENCES

1. Allen, P. A. & Kabat, E. A.: Immunochemical studies in blood groups. XXIII. Studies on cross reactivity of untreated and partially hydrolyzed blood group A, B, and O (H) substances with type XIV antipneumococcal horse sera. J Immunol *82:* 358, 1959.
2. Coombs, R. R. A., Bedford, D. & Rouillard, L. H.: A and B blood group antigens on human epidermal cells, demonstrated by mixed agglutination. Lancet *i:* 461, 1956.
3. Fisher, J. P.: Soluble substances of human stratum corneum I-immunochemical and immunologic study. J Invest Dermatol *44:* 43, 1965.
4. Gardas, A. & Koscielak, J.: A, B and H blood group specificities in glycoprotein and glycolipid fractions of human erythrocytes membranes. Absence of blood group active glycoproteins in the membranes of non-secretors. Vox Sang *20:* 137, 1971.
5. Grob, P. J. & Inderbitzin, T. M.: Pemphigus antigen and blood group substances A and B. J Invest Dermatol *49:* 295, 1967.
6. Hakamori, S. & Kobata, A.: Blood group antigens. *In* The Antigens, Vol. II (ed. M. Sela). pp. 79–140. Academic Press, New York, 1974.
7. Heaphy, M. R. & Winkelmann, R. K.: Preparation and ultrastructural features of human cutaneous basement membrane-anchoring fibril complex. Clin Res *24:* 263A, 1976.
8. Holborow, E. J., Brown, P. C., Glynn, L. E., Hawes, M. D., Gresham, G. A., O'Brien, T. F. & Coombs, R. R. A.: The distribution of the blood group A antigen in human tissues. Br J Exp Pathol *41:* 430, 1960.
9. Johnson, G. D. & Holborow, E. J.: Immunofluorescence. *In* Handbook of Experimental Immunology, 2nd ed., vol. 1 (ed. D. M. Weier). pp. 18.1. Blackwell Scientific Publications, Oxford, 1973.
10. Kabat, E. A.: Blood Group Substances, pp. 56. Academic Press, New York, 1956.
11. Kabat, E. A.: Immunochemical studies on the carbohydrate moiety of water soluble blood group A, B, H, Le^a, Le^b substances and their precursor I antigens. Advances in Chemistry, Series *117:* 334, 1973.
12. Matsumoto, I. & ●sawa, T.: Purification and characterization of an anti-H (●) phytohemagglutinin of Ulex Europeus. Biochim Biophys Acta *194:* 180, 1969.
13. Nelken, D., Gurevitch, J. & Neuman, Z.: A and B antigens in the human epidermis. J Clin Invest *36:* 749, 1957.
14. Olson, K., Biberfeld, G. & Fagraeus, A.: Blood group antibodies as a source of error in the diagnosis of pemphigus by indirect immunofluorescence. Acta Dermatovener (Stockholm) *52:* 389, 1972.
15. Ouchterlony, Ö.: Diffusion-in-gel methods for immunological analysis. Prog Allergy *5:* 1, 1958.
16. Schiff, F. & Sasaki, H.: Der Diastasegehalt des Liquor cerebrospinalis bei Syphilis. Klin Wochenschr *34:* 1426, 1932.
17. Szulman, A. E.: The histological distribution of blood group substances A and B in man. J Exp Med *111:* 785, 1960.
18. Watkins, W. M. & Morgan, W. T. J.: Role of O-β-D-galactopyranosyl-(1→4)- *N*-acetyl-D- glucosamine as inhibitor of precipitation of blood group substances by anti-type XIV pneumococcus serum. Nature *178:* 1289, 1956.
19. Unis, E. & Unis, J. J.: Cell antigens and cell specialization. III. On the H antigen receptors of human epidermal cells. Blood *22:* 750, 1963.

Received October 22, 1976

R. E. Jordon, M.D.
Mayo Clinic
Rochester, Minnesota 55901
USA