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Red Cell H-Deficient, Salivary ABH Secretor Phenotype of Reunion Island. Genetic Control of the Expression of H Antigen in the Skin

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Based on the genetic model proposing that *H* and *Se* are two structural genes, we predicted that the red cell H-deficient, salivary ABH secretor phenotype should be found on Reunion island, where a large series of H-deficient non-secretor families have been previously described. Two such Reunion individuals are now reported. POU [A_h, Le(a-b+), secretor of A, H, Le^a and Le^b in saliva] and SOU [O_h, Le(a-b+), secretor of H, Le^a and Le^b in saliva]. Both are devoid of H α-2-fucosyltransferase activity in serum. In addition, the preparation of total non-acid glycosphingolipids from plasma and red cells of POU revealed the type 1 ALe^b heptaglycosylceramide and small amounts of the monofucosylated type 1 A hexaglycosylceramide. Both glycolipids possess an H structure probably synthesised by the product of the *Se* gene. No other blood group A glycolipids, with types 2, 3 or 4 chains, normally present in the presence of the product of the *H* gene, were found on red cells or plasma of POU.

The *H*, *Se* and *Le* genetic control of the expression of ABH and related antigens in different tissue structures of the skin is described in 54 H-normal individuals of known ABO, secretor and Lewis phenotypes; in one red cell H-deficient salivary secretor (SOU); and in one H-deficient non-secretor (FRA). Sweat glands express ABH under the control of the *Se* gene. Sweat ducts express ABH under the control of both *H* and *Se* genes and Lewis antigens under the control of *Le* and both *H* and *Se* genes. Epidermis, vascular endothelium and red cells express ABH under the control of the *H* gene. The products of *H* and *Se* genes are usually expressed in different cells. However, the results illustrate that in some structures, like the epithelial cells of sweat ducts, both the products of *H* and *Se* genes can contribute to the synthesis of the same Le^b structure.

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The first genetic model for *H* and *Se* genes assumed that *H* was a structural gene coding for an α-2-fucosyltransferase and *Se* was a regulatory gene controlling the expression of *H* in exocrine secretions (reviewed in [1]).

Later on, we proposed [2] another genetic model assuming that *Se* and *H* are two distinct structural genes coding for two different α-2-fucosyltransferases, using preferentially type 1 and type 2 substrates, respectively. Since then, evidence for the existence of at least two different α-2-fucosyltransferases has been reported by different laboratories [3-5]. If *Se* and *H*, indeed, are two distinct genes which do not interact at the phenotypic level, then the linkage analysis of *H* and *Se* in the H deficient families demonstrates that they are closely linked [2, 6]. Therefore, since the *Se* locus is part of a large linkage group (familial hypercholesterolemia, Lewis blood group, complement component 3, Landsteiner-Wiener blood group, peptidase D, dystrophia myotonia, ABH secretion, apolipoprotein C-III, apolipoprotein E and Lutheran blood group) which has been assigned to chromosome 19 [7], the *H* locus is also expected to be on chromosome 19.

Our two structural genes model postulated the existence of both secretors and non-secretors among H-deficient individuals [6]. A few red cell H-deficient salivary ABH secretor individuals have already been reported by different authors (reviewed in [2]), but they are, by far, less numerous than the H-deficient non-secretors. Furthermore, in a large series of H-deficient families from Reunion [6, 8-10], the 42 individuals of Reunion phenotypes and the eight individuals of Bombay phenotype, were all non-secretors of ABH in saliva (Reunion is a French island located in the archipelago of Mascareignes in the Indian Ocean, 700 km east of Madagascar [8]).

The red cell H-deficient salivary ABH secretors have either low titres of anti-H, anti-HI or no anti-H at all ([11], reviewed in [12]). This is in contrast with H-deficient non-secretors who have generally strong anti-H activity in serum. Therefore, since the detection of H-deficient phenotypes is mainly based on the finding of irregular serum agglutinins, with anti-H activity, the low proportion of ABH secretors found among red cell H-deficient individuals could be due to red cell H-deficient salivary secretors, with low or no detectable anti-H, being missed by routine ABO typing. Consequently, a special effort to detect red cell H-deficient salivary secretor individuals on Reunion island has now been performed, by looking for the absence of H antigen on red cells and H enzyme in serum.

The present work describes the first two red cell H-deficient salivary ABH secretor individuals found on Reunion island and compares the tissue distribution of H antigens in skin, red cells and vascular endothelium with that of normal H controls. Furthermore, the glycosphingolipid-based blood group A expression from red cells and plasma from one of the red cell H-deficient salivary secretor individuals (blood group A) is compared with that of a normal, non-H-deficient control.

Materials and Methods

ABH Agglutination

Serological tests were performed on freshly washed red cells with commercial anti-ABH reagents (CNTS Institute, Paris, France). The presence of H antigen was further tested

with another human anti-H serum (CTS, Nantes, France), sera from known H-deficient non-secretors of Bombay and Reunion phenotypes [6] and purified lectin 1 from *Ulex europaeus* (Vector Laboratories, Burlingame, CA, USA).

Lewis and Secretor Phenotypes

Le^a and Le^b erythrocyte antigens were tested by agglutination of papain-treated red cells with goat antisera (Ortho Diagnostics, Raritan, NJ, USA) and purified mouse monoclonal antibodies (Chembiomed, Edmonton, Canada). Donors with Le(a+b-) erythrocytes were considered non-secretors of ABH, and donors with Le(a-b+) erythrocytes were considered secretors of ABH antigens. The salivary ABH secretor phenotype of Le(a-b-) individuals was determined either by inhibition of ABH hemagglutination with the saliva of donors or by immunofluorescent staining of sections of liver and parotid glands from necropsic samples with *Ulex europaeus* lectin 1.

Inhibition of Hemagglutination

Saliva samples were collected and heated in a boiling water bath for 10 min within 2 h of collection. The boiled salivas were clarified by centrifugation and stored at -20°C until used.

Hemagglutination of ABO red cells with the corresponding ABH reagents, diluted to the last point giving strong agglutination, was inhibited with saliva samples diluted 1 to 10.

Anti-ABH Serum Agglutinins

The presence of anti-A, anti-B, anti-H and anti-HI activity in serum of H-deficient individuals was tested in 10 mM sodium phosphate, 0.14 M sodium chloride, pH 7.2 (PBS) with fresh erythrocytes of known ABHI phenotypes. The presence of anti-H and anti-HI antibodies was also tested on papain-treated red cells.

Fucosyltransferase Assays

The α-2-L- and α-3-L-fucosyltransferase activities [13] were tested in serum of controls and red cell H-deficient non-secretor and secretor donors.

The reaction mixture for α-2-L-fucosyltransferase contained in a total volume of 50 μl: serum 25 μl; Tris-HCl, pH 7.2, 1.0 μmol; $MgCl_2$, 1.0 μmol; ATP 0.25 μmol; GDP-[^{14}C]fucose (210 μCi/μmol, NEN Chemicals, Frankfurt, W. Germany), 620 pmol; phenyl-β-D-galactoside, 0.16 μmol (Koch-Light Laboratories, Colnbrook, Berks, UK). After incubation for 24 h at 37°C, the reaction products were separated by paper chromatography in ethyl acetate/pyridine/water, 10/4/3 vol. The ^{14}C-labeled products were localized using a radiochromatogram scanner (Packard 7201) and quantified by liquid scintillation counting. The product was characterized by its chromatographic mobility using fucose as a reference (K_{fuc} = 1.5).

The reaction mixtures for α-3-L-fucosyltransferase were similar except that $MgCl_2$ was replaced by $MnCl_2$, 1.0 μmol; and phenyl-β-D-galactoside by *N*-acetyllactosamine, 0.23 μmol (Sockerbolaget, Arlöv, Sweden). The products were separated in the same solvent.

The specific product was characterized by its mobility using lactose as a reference (R_{lac} = 0.75).

Glycosphingolipids and the Antibody Overlay Technique on Thin-layer Plates

Total non-acid glycosphingolipids from red cells and plasma were isolated as described [14]. Identical series of the glycosphingolipid fractions were applied to a high performance thin-layer plate (Merck Si-60 on aluminium sheets) and developed in chloroform/methanol/water, 60/35/8 by vol. After chromatographic development, the thin layer plate was divided into several identical sections. One section was stained with anisaldehyde [14]. The antigenic reactivity of the separated glycolipids was tested on the remaining sections, using a modification [15] of the method of Magnani *et al.* [16]. The plates were treated with primary monoclonal antibodies followed by an appropriate anti-immunoglobulin ^{125}I-labeled secondary antibody. Plates were dried and autoradiographed. The identity of the different bands indicated from the red cell fraction was based on authentic standards prepared and structurally characterized at the Department of Medical Biochemistry in Göteborg [17] and by comparison with the results of Clausen *et al.* [18]. The identity of blood group A glycosphingolipids in plasma of an A_1 Le(a-b+) secretor and an A_1 Le(a-b-) secretor has been the subject of earlier papers [19-20].

Immunofluorescence

Skin biopsies from the H-deficient individuals SOU [O_h Le(a-b+), secretor of H in saliva] and FRA [B_h Le(a+b-), non-secretor of B or H in saliva] and skin samples from 54 H-normal, post-mortem forensic cases, were fixed in 4% paraformaldehyde (Bouin's fixative should not be used for fluorescent studies of the skin, because it induces a strong non-specific positive fluorescent reaction in the cytoplasm of epithelial cells). Paraformaldehyde fixed biopsies were paraffin embedded and processed for indirect immunofluorescence after deparaffination.

Monoclonal and polyclonal anti-A,B and H [21] and anti-Lewis [22, 23] affinity purified reagents, at 5 to 20 μg/ml were incubated on the slides for 30 min in a moist chamber. After washing, the corresponding FITC labeled anti-Ig (Pasteur Diagnostics, Marnes-la-Coquette, France) and TRITC labeled *Ulex europaeus* lectin 1 (Vector) were incubated for another 30 min. The slides were then washed and mounted with one drop of *p*-phenylenediamine (1 mg/ml in 90% glycerol brought to pH 8 with sodium carbonate buffer). This mounting solution diminishes the photobleaching of fluorescein [24] and induces a brown-yellow fluorescent counterstain of nuclei, that is very helpful in the identification of the FITC and TRITC negative tissue structures [25]. Simple and double superimposed pictures with the filter sets for fluorescein and rhodamine were taken in a Leitz epifluorescence microscope, equipped with a Ploemopak 2.3 and an exciting source of 200 W from a high pressure mercury vapour lamp (HBO), as previously described [26].

Results

Red Cell and Saliva Phenotypes

During the last three years, a special effort has been made in the Transfusion Centre of

Table 1. Serum α-2- and α-3-fucosyltransferase activities expressed as c.p.m. of [^{14}C]fucose incorporated in the phenyl-β-D-galactoside and *N*-acetyllactosamine acceptors, respectively.

Red cell phenotype	Serum fucosyltransferases	
	α-2 or H	α-3 or X
A_h Le(a-b+) POU)	0	5300
O_h Le(a-b+) (SOU)	0	17300
A Le(a-b+)	4700	5800
O Le(a-b+)	5600	10400

Saint Denis on Reunion island to detect individuals with red cell H-deficient salivary ABH secretor phenotype. Two such individuals were found.

The first red cell H-deficient salivary secretor (POU), was found by routine blood group typing, performed prior to her first pregnancy, because she had irregular anti-H activity in her serum. Her erythrocytes were not agglutinated by any of the anti-H reagents tested, but were agglutinated by some anti-A sera (A_h Reunion phenotype). In contrast with the previous H-deficient non-secretors, who were all Le(a+b-) or Le(a-b-), POU had Le(a-b+) red cell phenotype and secreted A, H, Le^a and Le^b antigens in saliva.

The red cells of the second individual (SOU) were not agglutinated by any of the anti-A, B and H reagents and were of Le(a-b+) phenotype. Her saliva had H, Le^a and Le^b antigens. This individual did not have irregular anti-H antibodies detectable by the routine agglutination tests. However, because of the suspicion of an H-deficient phenotype, a more thorough study of her serum revealed a very weak anti-HI antibody only detectable on papain treated erythrocytes. Therefore, these two individuals had the predicted red cell H-deficient, salivary ABH secretor, Reunion phenotype [6]. Similar phenotypes previously found in other areas of the world have been called "para-Bombay", but this term is rather ambiguous since it has also been used for salivary non-secretors with small amounts of H on red cells [12].

Serum Fucosyltransferases

Serum α-2- and α-3-fucosyltransferase activities were determined for both POU and SOU. As expected, both had normal α-3-fucosyltransferases and did not show α-2-fucosyltransferase activity (Table 1). The conditions of the present assay did not allow detection of the small amounts of α-2-fucosyltransferase previously reported for other red cell H-deficient salivary ABH secretors [26].

Glycosphingolipids

Plasma contains type 1 glycosphingolipids synthesized under the control of the products of the Lewis and secretor genes [27]. These plasma glycosphingolipids are also found on the red cell membrane and are assumed to be acquired from plasma, since red cells change their Lewis phenotype after transfer into solutions of purified glycosphingolipids [28] or into human recipients [29] with different Lewis or secretor phenotypes. For normal blood group A non-H-deficient individuals, a blood group A

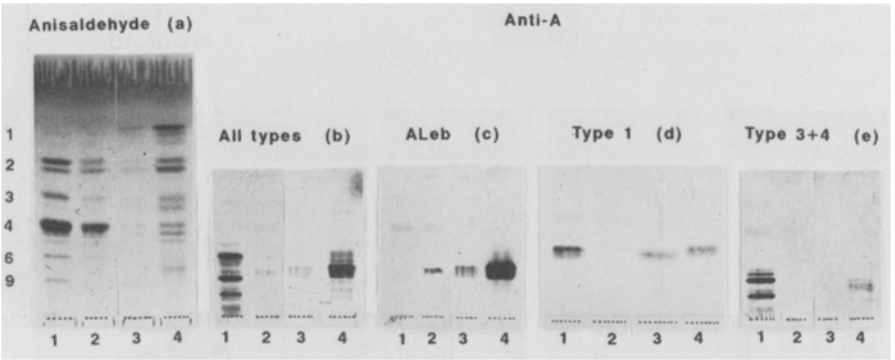

Figure 1. Thin-layer chromatography with anti-A antibody staining of total non-acid glycosphingolipid fractions from erythrocyte membranes (lanes 1 and 2) and plasma (lanes 3 and 4) from different blood group A individuals. The thin-layer chromatogram was developed in chloroform/methanol/water, 60/35/8 by vol. The numbers to the left indicate the approximate number of sugar residues in the carbohydrate chain of each glycolipid band.
Lane 1 = red cells of an H-normal, A Le(a-b-) secretor control (*A/A* or *O*, *H/H* or *h*, *Se/Se* or *se*, *le/le*).
Lane 2 = red cells of the H-deficient, A_h Le(a-b+) secretor donor POU (*A/A* or *O*, *h/h*, *Se/Se* or *se*, *Le/Le* or *le*).
Lane 3 = plasma of the same red cell H-deficient salivary ABH secretor donor POU.
Lane 4 = plasma of an H-normal, A Le(a-b+) secretor control (*A/A* or *O*, *H/H* or h, *Se/Se* or *se*, *Le/Le* or *le*).
The same sequence of samples, lanes 1-4, was applied repetitively on the same thin-layer plate. After chromatographic development, the thin layer plate was divided into identical sections. One section (a) was visualized with the anisaldehyde reagent [14]. The remaining 4 sections (b, c, d and e) were subjected to the antibody overlay technique followed by autoradiography [15, 16]. The figure shows the autoradiograms. The following mouse monoclonal antibodies were used: (b) A581 (Dakopatts), which is an anti-A antibody reacting with the blood group A terminal trisaccharide irrespective of core chain type (1 to 4), mono- and di-fucosyl compounds [43]; (c) HH3 which is an anti-ALe^b antibody, specific for a difucosyl blood group A determinant carried by a type 1 carbohydrate core chain [33]; (d) AH21 which is an anti-A antibody specific for monofucosyl blood group A determinant carried by type 1 carbohydrate core chain; (e) KB-26.5 which is an anti-A antibody, specific for a blood group A determinant carried by a type 3 or 4 carbohydrate core chain [44]. Plasma and red cells of the H-deficient donor POU seem to contain the same ALe^b glycolipid. Plasma contains also small amounts of an A type 1 glycolipid antigen. All other blood group A glycolipids (types 2, 3 and 4, found in the normal controls) seem to be absent.

hexaglycosylceramide with a type 1 carbohydrate chain has been identified in plasma of an A_1 Le(a-b-) secretor [19]. A blood group A difucosyl heptaglycosylceramide with a type 1 chain (A Le^b) has been identified in plasma of an A_1 Le(a-b+) secretor individual [20]. In addition, erythrocytes have intrinsic type 2 [30], type 3 [31] and type 4 [32] chain blood group A glycolipids. The main constitutive blood group A glycolipids of the red cell membrane seem, however, to be type 2 and type 3 [18], which are both supposed to be under the control of the product of the *H* gene.

Total non-acid glycosphingolipids of plasma and red cell membranes were prepared from 50 ml of whole blood of the A_h Le(a-b+) Reunion donor (POU) and were analyzed by the antibody overlay technique on thin-layer chromatograms, using different monoclonal anti-A reagents. Fig. 1 illustrates the probable lack of blood group A type 2, 3 and 4 glycolipids from both red cells and plasma of this A_h Le(a-b+) donor. The main blood group A active glycolipid found both in the plasma and the red cell fraction had

an R_F-value identical with the difucosylated type 1 chain blood group A heptaglycosylceramide or A Le^b (Fig. 1c, lane 4), identified in plasma of non-H deficient A Le(a-b+) secretors [20]. It was revealed with the blood group A specific reagent (Dakopatts A581) (Fig. 1b). It was also strongly recognized by the A Le^b specific monoclonal antibody HH3 [18] (Fig. 1c). It is assumed that the A Le^b heptaglycosylceramide found on erythrocytes of this A_h Le(a-b+) donor is adsorbed from plasma in a manner similar to that which occurs in non-H-deficient individuals [19, 27, 33]. The presence of A Le^b heptaglycosylceramide on red blood cells of the A_h Le(a-b+) secretor should thus be under the control of the products of the *Le* and *Se* genes and independent of the expression of the *H* gene. In addition, small amounts of a glycolipid which is recognized by the anti-A type 1 specific antibody AH21 [33], were found in plasma of the H-deficient A Le(a-b+) secretor (Fig. 1d, lane 3; POU). Such an antigen is also found in small amounts in plasma from non-H-deficient A Le(a-b+) secretors (Fig. 1d, lane 4) and has been found in much larger amounts in a normal A_1 Le(a-b-) secretor [19].

Immunofluorescence

A skin biopsy was obtained from the O_h Le(a-b+) Reunion donor (SOU) and its staining with anti-ABH, anti-Le^a and anti-Le^b antibodies was studied in parallel to a skin biopsy of a Reunion red cell H-deficient salivary ABH non-secretor donor B_h Le(a+b-) (FRA, II-17 of pedigree Pa4 in [8]) and skin biopsies of 54 ABH normal controls.

In the skin, two different genetic controls of the expression of ABH antigens can be distinguished by immunofluorescent staining. Red cells, vascular endothelium and epidermal cells were not stained with anti-ABH in *hh* individuals, whereas they were always positive in *HH* or *Hh* individuals irrespective of the secretor genotype of the tissue donor (Fig. 2a). On the other hand, acinar cells of the coiled sweat glands were always positive with the corresponding anti-A, B or H on biopsies of secretor individuals (*SeSe* or *Sese*) and negative on non-secretors (*se/se*) irrespective of the H genotype of the tissue donor (Table 2).

The sweat ducts had a particular staining pattern. They expressed only small amounts of B antigen in the biopsy of FRA (Fig. 2b) and normal amounts of ABH in the other donors irrespective of their H and secretor phenotypes, suggesting that these epithelial cells can express H antigen under the control of the products of both the *H* and *Se* genes. Therefore, the epithelial cells of sweat ducts seem to have the properties of both the epithelial cells of the epidermis (under the control of the *H* gene) and the acinal cells of the coiled sweat glands (under the control of the *Se* gene). In addition, sweat ducts were the only skin structures which expressed large amounts of Lewis antigens. The Le^a and Le^b antigens were present in all Lewis positive donors provided they had one active *H* or *Se* gene. The H-deficient non-secretor donor (FRA) expressed only Le^a antigen, whilst the red cell H-deficient salivary secretor donor (SOU) expressed both Le^a and Le^b. As expected, the sweat ducts of Lewis negative individuals (*le/le*) were not stained with any of the anti-Lewis reagents. However, anti-Le^b antibodies were positive in sweat ducts of H-normal secretors and non-secretors, confirming that both the products of the *H* and *Se* genes can contribute to the synthesis of the Le^b antigen in this particular skin structure. Fig. 2c illustrates the anti-Le^b positive reaction (green) on the sweat ducts of the skin of an H-normal *H/H* or *h*, *se/se*, *Le/Le* or *le*, individual.

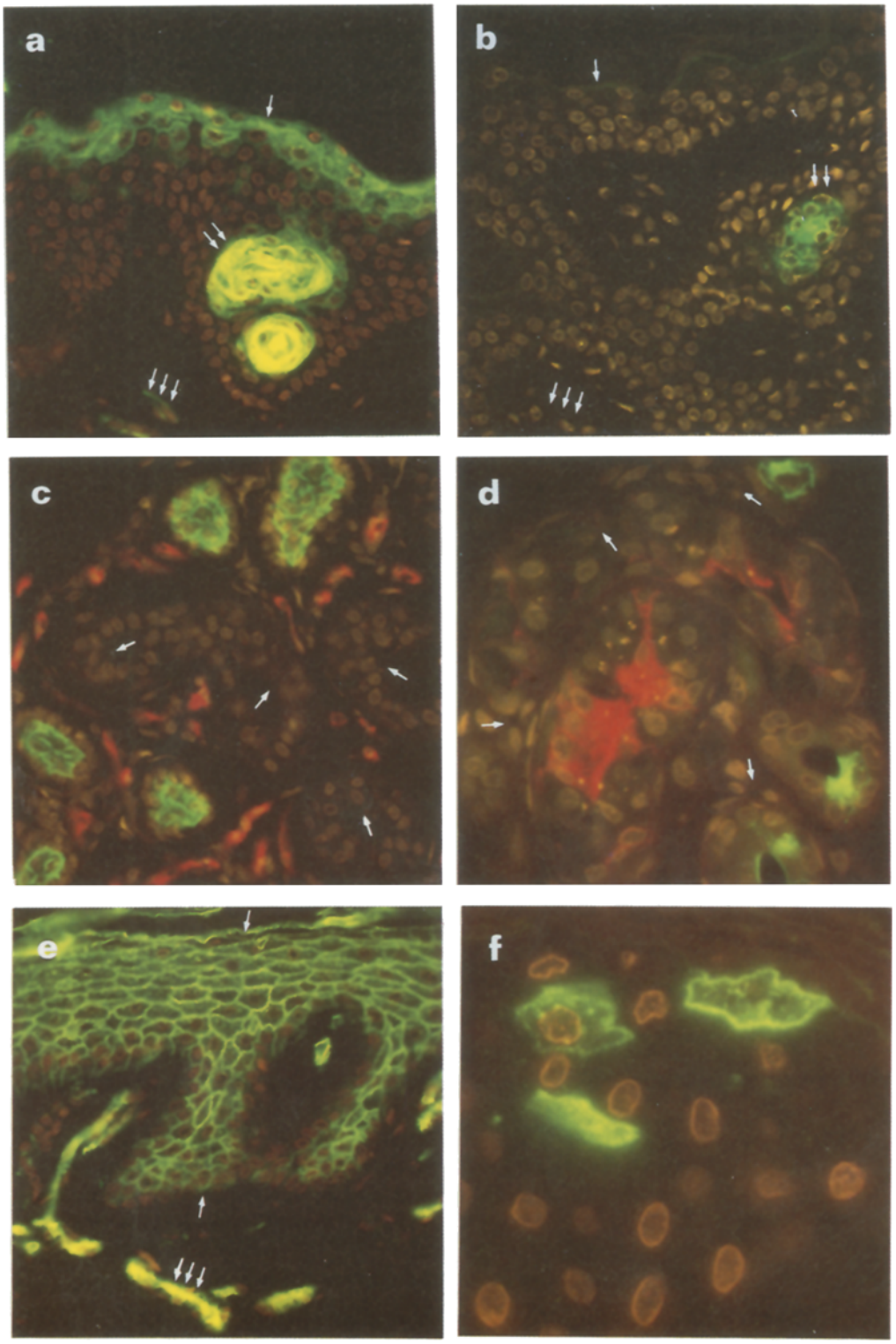
a
b
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Table 2. Immunofluorescent staining of tissue structures on paraffin embedded skin biopsies of individuals of different ABO, H, secretor and Lewis genotypes with anti-A, anti-B, anti-H, anti-Le^a and anti-Le^b specific reagents.

Genotype				Number tested	Immunofluorescent staining with anti-ABH and anti-Lewis reagents.[a]						
ABO	Hh	Sese	Lele		Red cells	Vascular endothelium	Stratum granulosum	Stratum spinosum	Sebaceous glands	Sweat glands[b]	Sweat ducts
B/-	*h/h*	*se/se*	*Le/-*	1 (FRA)	-[a]	-	-	-	-	-	Bw,Le^a
O/O	*h/h*	*Se/-*	*Le/-*	1 (SOU)	-	-	-	-	-	H	H,Le^a,Le^b
A/-	*H/-*	*se/se*	*Le/-*	3	A,H	A,H	A,H	H	H	-	A,H,Le^a,Le^b
A/-	*H/-*	*Se/-*	*Le/-*	16	A,H	A,H	A,H	H	H	A,H	A,H,Le^a,Le^b
A/-	*H/-*	*Se/-*	*le/le*	2	A,H	A,H	A,H	H	H	A,H	A,H
A/-	*H/-*	*se/se*	*le/le*	2	A,H	A,H	A,H	H	H	-	A,H
A/B	*H/-*	*se/se*	*Le/-*	1	A,B,H	A,B,H	A,B,H	H	H	-	A,B,H,Le^a,Le^b
A/B	*H/-*	*Se/-*	*Le/-*	1	A,B,H	A,B,H	A,B,H	H	H	A,B,H	A,B,H,Le^a,Le^b
B/-	*H/-*	*se/se*	*Le/-*	1	B,H	B,H	B.H	H	H	-	B,H,Le^a,Le^b
B/-	*H/-*	*Se/-*	*Le/-*	4	B,H	B,H	B,H	H	H	B,H	B,H,Le^a,Le^b
B/-	*H/-*	*Se/-*	*le/le*	3	B,H	B,H	B,H	H	H	B,H	B,H
B/-	*H/-*	*se/se*	*le/le*	1	B,H	B,H	B,H	H	H	-	B,H
O/O	*H/-*	*se/se*	*Le/-*	5	H	H	H	H	H	-	H,Le^a,Le^b
O/O	*H/-*	*Se/-*	*Le/-*	13	H	H	H	H	H	H	H,Le^a,Le^b

[a] -; no staining. A,B,H, Le; positive staining with the corresponding reagent. Bw; only weak staining with some anti-B of broad specificity.
[b] only a few isolated acinar cells were positive with anti-Lewis reagents (not shown).

Figure 2. Immunofluorescent staining of paraffin embedded skin biopsies of H-normal and H-deficient individuals stained with anti-A, B, H and Lewis reagents.
(a) Thin skin of an H-normal, *B/B* or *O*, *H/H* or *h*, *se/se*, *le/le* individual, stained with polyclonal, affinity purified, goat anti-B antibodies. The epithelial cells of the stratum granulosum (arrow), the sweat ducts (double arrow) and the vascular endothelial cells (triple arrow) are positive. Magnification × 300.
(b) Thin skin of the Reunion H-deficient non-secretor individual FRA, (*B/B* or *O*, *h/h*, *se/se*, *Le/Le* or *le*), stained with the same anti-B as in (a). Only the sweat duct (double arrow) is positive. The stratum granulosum (arrow) and vascular endothelial cells (triple arrow) are negative. Magnification × 300.
(c) Coiled sweat gland in the dermis of an H-normal, *O/O*, *H/H* or *h*, *se/se*, *Le/Le* or *le*, individual double stained with anti-Le^b and *Ulex europaeus* lectin 1. The inner epithelial cells of sweat ducts are strongly stained with anti-Le^b (green). The vascular endothelial cells are positive with *Ulex europaeus* (red). The acinar cells of coiled sweat glands are negative (arrows). Magnification × 300.
(d) Sweat gland of the Reunion H-deficient donor SOU, (*O/O*, *h/h*, *Se/Se* or *se*, *Le/Le* or *le*), double stained with anti-Le^a and the lectin 1 of *Ulex europaeus*. The Le^a antigen (green) is present in cells of sweat ducts, like Le^b in (c). However, acinar cells of sweat glands and vascular endothelium have an H staining pattern different from that illustrated in (c). Indeed, *Ulex europaeus* reveals the presence of H antigen (red) in the acinar cells of coiled sweats glands, in accordance with the secretor phenotype of this donor, whilst vascular endothelial cells of the numerous intertubular capillaries are negative (arrows), in agreement with the H-deficient phenotype of this individual (compare (c) and (d) for the red stain of capillaries and acinar cells). Magnification × 480.
(e) Thick skin of the palm of the hand of an H-normal, *O/O*, *H/H* or *h*, *Se/Se* or *se*, *Le/Le* or *le*, individual stained with polyclonal, affinity purified, goat anti-H antibodies. All layers of epithelial cells of the epidermis are positive (arrows). Sweat ducts (not shown) and vascular endothelial cells (triple arrow) are also positive. Magnification × 300.
(f) Thick skin of the thumb of an H-normal, *A/A* or *O*, *H/H* or *h*, *Se/Se* or *se*, *Le/Le* or *le*, individual stained with polyclonal, affinity purified, rabbit anti-A, antibodies. Only a few cells in the upper layer of the stratum granulosum are positive. Magnification × 1500.

Faint staining with anti-Lewis antibodies was observed in some acinar cells of some coiled sweat glands. However, since this stain was weak and irregular, it is not reported in Table 2. This reaction was found in about 20% of the Lewis positive individuals (*Le/Le* or *le*, *Se/Se* or *se*; or *Le/Le* or *le*, *se/se*) and was never found in Lewis negative samples (*le/le*). Fig. 2d illustrates the presence of positive cells in the sweat ducts with anti-Le^a (green) and the absence of this antigen on the acinar cells of the coiled sweat glands of the *h/h*, *Se/Se* or *se*, *Le/Le* or *le* donor (SOU). It is interesting to note that this red cell H-deficient salivary secretor donor expressed H antigen in acinar cells of her sweat glands (red) in accordance with her salivary secretor phenotype and did not have any positive reaction on the vascular endothelial cells, with the same anti-H reagent, in accordance with her red cell H-deficient character. The epithelial cells of sweat ducts of this individual were also positive with anti-Le^b (not shown).

By immunofluorescence, Lewis antigens have not been detected on epithelial cells of the epidermis, red cells, vascular endothelial cells or the epithelial cells of sebaceous glands [34, 35]. Small amounts of H antigen were detected in sebaceous glands of all H normal individuals, irrespective of their secretor status (Table 2).

The cytoplasm of epidermal cells was not stained with the anti-blood-group reagents. The expression of ABH antigens is, on the contrary, concentrated on the cellular membranes (Fig. 2a, e, f). In thin skin, the three ABH antigens are expressed on cell membranes of the few upper layers of the stratum granulosum, right under the dead cells of the stratum corneum (Fig. 2a). Conversely, in thick skin (palm of hands and sole of feet), the H antigen is also expressed in the cell membranes of all the deeper layers (stratum granulosum, spinosum and germinativum) of the epidermis (Fig. 2e). The expression of A or B antigens, in this thick skin, is restricted to some isolated cells in the upper layer of the stratum granulosum (Fig. 2f).

Discussion

The counterstain of nuclei from non-fluorescent stained cells with *p*-phenylenediamine allowed us to dissect the rather complex pattern of genetic control of the expression of ABH and related antigens in the skin.

According to our two structural gene model, acinar cells of sweat glands expressed large amounts of ABH under the control of the product of the *Se* gene and very little (faint and occasional) Lewis antigens. The epithelial cells of sweat ducts expressed ABH and Lewis antigens under the control of *Le* and both *H* and *Se* genes. The surface of the epidermis expressed only ABH antigens under the control of the *H* gene. Vascular endothelium and red cells had also ABH antigens under this same *H* genetic control. A similar *H* genetic control was found in deep layers of the epidermis and sebaceous glands, but no A or B antigens were found at this level [35].

Le^a and Le^b antigens are easily detected in some tissue structures such as the sweat ducts. However, they are difficult to detect in secretor acinar cells of sweat glands and have never been detected, by immunohistochemistry, on cells such as vascular endothelium or erythrocytes [22, 34, 35]. Since both Le^a and Le^b specificities can be detected by agglutination on red cells, we have to assume that some steps of the preparation of tissues for the immunohistochemical reactions, either wash out, destroy or mask part of the Lewis antigens. This phenomenon may be related to the nature of

the carrier of the Lewis carbohydrate antigens. The Lewis glycosphingolipids, which are known to be adsorbed on blood cells, might be particularly sensitive to aggressive histochemical techniques, which frequently use organic solvents; whereas Lewis glycoproteins synthesized by epithelial cells seem to resist this treatment better.

The finding that the products of both *H* and *Se* genes can contribute *in vivo* to the synthesis of the type 1, Le^b antigen is in agreement with previous *in vitro* work showing that serum α-2-fucosyltransferases can use both type 1 and type 2 acceptor substrates [36] and brings a new interpretation for the finding of Le^b antigen in some tissues of non-secretor (*se/se*, *Le/Le* or *le*) individuals. Indeed, Limas [37] and Orntoft [38] have recently reported strong expression of Le^b antigen in urinary epithelium of all the Le(a+b-) donors tested. Furthermore, small amounts of Le^b antigen in deep glands of the digestive mucosa of Lewis positive non-secretor individuals [34, 39] were previously assumed to be secondary to cross-reactions of the anti-Le^b serum. However, its presence can also be explained by this new concept of an *H-Le* epistatic interaction, in addition to the classical *Se-Le* epistatic interaction, which produces the majority of the Le^b antigen in the digestive mucosa. A similar *H-Le* epistatic interaction has been posulated by Dabelsteen (personal communication) in order to interpret the presence of Le^b antigen in the epithelial cells of salivary ducts of non-secretor, Lewis positive individuals.

The immunochemical characterization of the glycosphingolipid fractions prepared from red cells and plasma is important and adds to the concept described in several ways. First, it is a defined molecular class of carbohydrate antigens (glycolipids) that is under study. Secondly, this molecular class may be lost during the histochemical technique. Thirdly, the identification of a glycosphingolipid-based carbohydrate antigen is based both on the specificity of the ligand-binding (monoclonal antibody) and chromatographic mobility. The same antigenic determinant may be present on different basic structures and thus chromatographically distinguishable. One interesting aspect of the glycosphingolipid studies is the apparent concomitant absence of both type 2 and type 3 chain blood group A antigens from the red cells of the A_h Le(a-b+) secretor individual (POU). However, the finding is in complete agreement with the data of Clausen *et al.* [40] showing that the biosynthesis of type 3 chain A is basically an elongation of the type 2 chain hexaglycosylceramide (repetitive A epitope). The genetic regulation of the addition of the second fucose in an α(1-2)-linkage is, however, not fully known at present. The *Se* gene seems to regulate the expression of type 3 chain A in epithelial tissues, while it is expressed independently of the secretor status on erythrocytes [40].

The apparent absence of type 4 chain A is also interesting. This antigen is a major A antigen in kidney and its expression has been suggested to be related to the presence of the *Se* gene [40, 41]. This is, however, not necessarily true in erythrocytes.

Overall, the results illustrate that, besides red cells [1] and platelets [42], the expression of some other tissue H antigens is also under the genetic control of the α-2-L-fucosyltransferase product of the *H* gene. Under the first genetic model, proposing that there is only *H* structural gene and *Se* is a regulatory gene for the expression of *H* in saliva [1], all H deficient individuals should be non-secretors of ABH in saliva. Therefore, the absence of red cell H deficient salivary ABH secretors in our first reports on Reunion island [6, 8, 9], created some concern among the supporters of the regulatory model for

Se and *H* genes, since no red cell H deficient salivary secretors were found in these original reports. (The finding of red cell H-deficient salivary secretor phenotypes on Reunion island clears these doubts and supports the two structural gene model for *H* and *Se* [2], since the red cell H-deficient salivary ABH secretor phenotype cannot be explained with the regulatory model, unless we accept the hypothetical existence of a third genetic locus (*Zz*), regulating the expression of H on red cells [11]. Our two structural gene model is the more economic interpretation of the available data. However, we have to admit that all data can also be interpreted with the previous and more complex model assuming that there is one structural *H* gene and two regulatory genes, *Se* and *Z*, one for the expression of H antigen in saliva and the other for the expression of H antigen on red cells.

Due to the theoretical importance of the existence of the red cell H-deficient salivary secretor phenotype on Reunion, a special effort was made to characterize thoroughly the Reunion red cell H-deficient salivary secretors found. In addition to the serological data, absence of α-2-fucosyltransferase from serum, absence of H dependent glycolipids from red cells and tissue distribution of H antigens, largely confirmed the H-deficient character of these secretor individuals. These findings are in good agreement with our prediction of the existence of H-deficient secretors on Reunion island [6], but they do not explain the low proportion of secretors found among H-deficient individuals. This problem could be due to linkage disequilibrium since *H* and *Se* are closely linked [2], but the ascertainment bias due to the low titres of anti-H antibodies found in red cell H-deficient salivary secretors does not allow determination of whether this phenomenon is only due to linkage disequilibrium or to ascertainment bias. Most probably, both factors are at play.

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