INFECTION AND IMMUNITY, Nov. 1990, p. 3807–3809
0019-9567/90/113807-03$02.00/0

Vol. 58, No. 11

Combined Inheritance of Epithelial and Erythrocyte Receptors for *Haemophilus influenzae*

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Received 30 May 1990/Accepted 8 August 1990

***Haemophilus influenzae* type b expressing fimbriae showed no adherence to buccal epithelial cells and no agglutination of erythrocytes from three AnWj-negative siblings in one family. Hemagglutination of erythrocytes from 13 AnWj-positive members of the same family and from 24 controls was normal, and *H. influenzae* adhered well to buccal epithelial cells from them. These data indicate that the expression of epithelial and erythrocyte receptors for *H. influenzae* is inherited concomitantly. Combined with previous data (L. van Alphen, J. Poole, L. Geelen, and H. C. Zanen, Infect. Immun. 55:2355–2358, 1987), the results show that the receptor molecules on the surfaces of the epithelial cell and the erythrocyte are different but that the binding sites for the fimbriae of *H. influenzae* are similar.**

Fimbriae of nontypeable as well as type b *Haemophilus influenzae* mediate bacterial adherence to oropharyngeal epithelial cells and to erythrocytes, yielding hemagglutination (HA) of the latter cells (12). The chemical structures of the epithelial cell and the erythrocyte receptors have not been resolved.

Erythrocytes with the AnWj-negative phenotype (previously referred to as Anton or Wj) lack the receptor for fimbriated *H. influenzae* (7, 11). The structural gene for this antigen has not been identified. Individuals with blood group Lu(a−b−) of the dominant type, who have the rare *In(Lu)* gene, have erythrocytes that do not express the AnWj antigen (4, 6, 7, 11). The inhibition of expression of the AnWj antigen on erythrocytes in Lu(a−b−) individuals is not always absolute, since with antibody adsorption-elution techniques low amounts of this antigen are sometimes detected (4). In addition, the AnWj antigen is absent on cells of a small number of individuals with anti-AnWj in their sera and on cord cells of all newborns (6, 7).

Previously, using data obtained from such AnWj-negative individuals, we concluded that the epithelial cell receptor and the erythrocyte receptor for *H. influenzae* fimbriae are expressed independently (10). This conclusion was based on the observation that *H. influenzae* adhered to buccal epithelial cells from healthy newborns and from an AnWj-negative individual with anti-AnWj in her serum. The erythrocytes of this individual were not agglutinated by fimbriated *H. influenzae*, and erythrocytes of newborns shifted from the HA-negative to the HA-positive phenotype between days 3 and 42 after birth.

In this communication, we report a different finding. Erythrocytes from three siblings who have the rare AnWj-negative blood group phenotype were not agglutinated by fimbriated *H. influenzae* and, unlike the above, their buccal epithelial cells also lacked the binding site for the fimbriae. In these individuals, however, the failure to express the AnWj antigen was based on a different mechanism from that described in our earlier publication. Here, the AnWj phenotype was recessive. On the basis of this observation, a hypothesis is presented for the relation between the erythrocyte and epithelial cell receptors.

Blood samples and buccal epithelial cells were obtained from 16 members of an Israeli Arab family. Relevant data are summarized in Table 1. Detailed characteristics of this family will be given elsewhere (J. Poole, C. Levene, M. Bennett, R. Sela, L. van Alphen, and P. Spruell, unpublished data). In addition, blood samples and buccal epithelial cells were obtained from two related Lu(a−b−) individuals of the *In(Lu)* dominant type [proposita III-1 and their sibling mentioned in reference 2, not to be confused with proposita III-1–6 in Table 1], with AnWj-negative erythrocytes (6). As controls, erythrocytes and buccal epithelial cells were collected from healthy adults. Samples, processed as described in detail before (10), were kept in medium consisting of Eagle minimal essential medium supplemented with Hanks salts, 10% fetal calf serum, 2 mM L-glutamine, 100 U of penicillin G per ml, 100 mg of streptomycin sulfate per liter, 1.25 U of Mycostatin per ml, and 1% dimethyl sulfoxide. They were frozen at −70°C until transported and then were sent by express mail from Israel to The Netherlands and the United Kingdom. Samples were analyzed immediately upon arrival. Storage of control buccal epithelial cells and of erythrocytes for 7 days at various temperatures between −70°C and room temperature did not affect adherence and HA. *H. influenzae* b strain 770235f$^+$, which expresses fimbriae, and its nonfimbriated variant, strain 770235, were used in HA and the adherence studies as described before (12).

The results obtained from the HA and the adherence experiments with the Arab family are summarized in Table 1. The three AnWj-negative family members had erythrocytes which were not agglutinated by the fimbriated *H. influenzae* bacteria and had buccal epithelial cells that did not bind the bacteria (less than one bacterium per cell). Erythrocytes from the AnWj-positive members of the family were agglutinated; their buccal epithelial cells, when available for assay, bound fimbriated bacteria (>50 per cell). The buccal epithelial cells of 10 healthy controls bound at least 5 (mostly >200) fimbriated bacteria of *H. influenzae* b strain 770235f$^+$ per buccal epithelial cell under the same experi-

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TABLE 1. AnWj blood group antigen expression, agglutination of erythrocytes (HA), and adherence to buccal epithelial cells by *H. influenzae* b strain 770235f+[a]

Family member	Erythrocytes		Adherence[b]
	AnWj antigen	HA[c]	
First generation (parents of proposita)			
I-1	+	+	NA[d]
I-2	+	+	NA
Second generation (siblings)			
II-1	+	+	+
II-2	−	−	−
II-3	+	+	+
II-4	+	+	NA
II-5	−	−	−
Ii-6	+	+	NA
II-7	+	+	+
II-8 (proposita)	−	−	−
Third generation (children of proposita II-8)			
III-1-6	+	+	NA

[a] Cells were obtained from 16 members of an Israeli Arab family.
[b] +, >50 bacteria per epithelial cell; −, <1 bacterium per cell.
[c] +, always immediate and can be seen with the naked eye; −, still negative after 1 h as observed by dark-field microscopy.
[d] NA, No cells available.

mental conditions. Bacteria of the nonfimbriated variant strain 770235 showed no adherence and caused no HA. The results obtained with cells from these controls correspond with those obtained previously by others and us (3, 5, 8, 9, 13). In the case of the Lu(a−b−) individuals, HA by fimbriated *H. influenzae* was negative as expected. In contrast, binding of these bacteria to their epithelial cells was observed. No HA or binding to epithelial cells by nonfimbriated bacteria occurred.

Hence, whereas previous data showed that individuals could have erythrocytes that were not hemagglutinated by fimbriated *H. influenzae* but whose epithelial cells did attach the bacteria, the current data show that, due to a different genetic mechanism, individuals can be negative for both HA and adherence. We demonstrated that expression of the AnWj blood group antigen, the erythrocyte receptor, and the epithelial cell receptor for *H. influenzae* has been inherited concomitantly in an Israeli Arab family, indicating a common genetic basis for the three phenotypes. In a separate paper, we show that the AnWj gene has a recessive mode of inheritance (J. Poole, C. Levene, M. Bennett, R. Sela, L. van Alphen, and P. Spruell, submitted for publication).

Despite a common genetic basis for the receptors on erythrocytes and epithelial cells for fimbriated *H. influenzae*, anti-AnWj antibodies inhibit HA but not adherence to epithelial cells from AnWj-positive adults (10). This indicates that (part of) the receptor molecule on erythrocytes and epithelial cells is different. This assumption is supported by the observation that the receptors on epithelial cells and erythrocytes are expressed independently. (i) The erythrocyte receptor is not expressed on cord erythrocytes, but the epithelial cell receptor is present at birth. On erythrocytes, the receptor and the AnWj antigen appear 3 to 42 days after birth. Expression of the receptor for fimbriated *H. influenzae* and the AnWj antigen is complete within 1 day (7). (ii) In some AnWj-negative individuals, the rare *In(Lu)* gene suppresses expression of the AnWj antigen strongly on erythrocytes of Lu(a−b−) individuals (4, 6). However, in these Lu(a−b−) individuals, adherence of *H. influenzae* to their buccal epithelial cells is still apparent. (iii) An individual with anti-AnWj was described who was AnWj negative concurrently but did express the epithelial cell receptor (10). Three years later, this individual (described as Kikk. in reference 6) was found to express AnWj on her erythrocytes, coinciding with disappearance of anti-AnWj in the plasma (unpublished observations). Apparently, the AnWj-negative phenotype was transient, indicating that the AnWj gene is present in this individual. So, since the aforementioned groups of individuals have some AnWj antigen on their erythrocytes, or have the ability to express it, they must have the structural gene for AnWj and thus the erythrocyte receptor (7, 11).

The simplest interpretation of the data is that the receptors for the fimbriae of *H. influenzae* on epithelial cells and erythrocytes are very similar but have different antigenic properties and that synthesis of the receptors is coded for by the gene which is not expressed in some of the family members described in this paper. The synthesis of the erythrocyte receptor is most likely suppressed in the *In(Lu)* Lu(a−b−) individuals, newborns, and the small number of individuals with anti-AnWj, who may not be *In(Lu)*. The receptors consist probably of a sugar moiety, since (i) *H. influenzae*-dependant HA and adherence to epithelial cells are not affected by proteolytic enzymes (4, 6, 11) and (ii) the AnWj antigen and the erythrocyte and epithelial cell receptors for *H. influenzae* were inactivated by mild oxidation with periodate (unpublished observation). These observations do not exclude that lipids are involved in bacterial binding, but treatment of these cells with chloroform-methanol or mild detergents did not reduce the binding activity of the cells (unpublished observations). In addition, sugar moieties are very common as receptors for various substrates, including hormones and surface molecules on cells, viruses, and bacteria (1). Similar sugar molecules often occur on different membrane components such as glycoproteins and glycolipids (1). These molecules may lack immunological cross-reactivity, especially when the sugar part is weakly antigenic.

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