DEVELOPMENT OF THE ISOSEROLOGICAL SYSTEM OF LEWIS DURING EMBRYOGENESIS

(UDC 612.118.22:611-013)

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Translated from Byulleten' Éksperimental Biologii i Meditsiny, Vol. 57, No. 5, pp. 62-64, May, 1964
Original article submitted April 12, 1963

The isoserologic system of Lewis differs essentially from many other isoserologic systems as follows: 1) the antigens Le (a+) and Le(b+) are apparently sera which become fixed on erythrocytes; 2) there is a relationship between these antigens in the blood of adult and the agglutinogens of the ABO system; group factors of the Lewis system cannot be analyzed genetically.

We have found no reports dealing with the antigens Le(a+) and Le(b+) in the blood of fetuses, although investigations of this kind have been carried out [3, 4]. Information concerning antigens of the Lewis system in the blood of the newborn are contradictory [2, 5-7].

In the present work we have made an attempt to discover the antigens Le(a+) and Le(b+) in the blood of human fetuses and in newborn infants.

EXPERIMENTAL METHOD

We studied the blood of fetuses produced by spontaneous abortion at the 17-30th week of pregnancy (length from sinciput to coccyx 15.5-29 cm) as well as blood from the umbilical cord of infants delivered at term, and blood of the parent. The erythrocytes were washed once with physiological saline, and a suspension of them was prepared in the same fluid. We used hetero-immune sera anti-Le^a and anti-Le^b obtained by L. K. Arzhelas [1]; these sera were obtained by immunization of goats against saliva obtained from humans whose blood belonged to group Le(a+b-) and Le(a-b+) (prepared by the Serum Department of the Institute of Forensic Medicine). If the sera contained complete antibodies, to one drop of a 1% suspension of erythrocytes two drops of sera were added, and the mixture was centrifuged for 4-5 min at 2,000 revs/min. It the sera contained incomplete antibodies, the erythrocytes were first treated with a 0.1% buffered solution of trypsin. To one drop of a 2% suspension of erythrocytes in physiological saline were added two drops of serum; the mixture was left for 1 h at room temperature, and then centrifuged for one minute at 1,000 revs/min. In both cases after centrifugation each test tube was shaken separately and the results of the agglutination reaction were studied with the naked eye, under a lens, and microscopically (on an object glass under a cover slip).

Before determination of the Lewis groups, the suspension of erythrocytes was examined microscopically in order that we could be sure of its suitability (absence of agglutination). The agglutinating power and specificity of the sera was determined systematically by including in the experiment blood of microdonors of groups Le(a+b-) and Le (a-b+).

To make a comparison of the Lewis groups with the extent of separation of the agglutinogens of the ABO system, we examined the saliva of the parent. The groups of the latter system were determined also in the saliva of certain of the newborn; the saliva was collected on gauze before the first feed. The adult saliva was centrifuged immediately after it had been obtained and then poured out onto gauze lying in several layers in sterile Petri dishes. With the saliva which had dried at room temperature we carried out the reaction of absorption of agglutinins with normal α and β sera (isosera) and with hetero-immune anti-O (H) serum prepared by immunization of a goat with

alcoholic dissenteric Grigor'eva-Shiga vaccine. The α and β sera were used in a titer of 1:16-1:32, and the anti-O (H) serum in a titer of 1:12-1:20. To 25 mg portions of gauze and saliva and to the same weight of gauze from the control portions without saliva 0.15 ml of serum were added. The absorption lasted 20-24 h at 4°. The results of the reaction were determined by titration of the absorbed and of the original α and β sera diluted a whole number of times and of the anti-O (H) sera diluted various amounts. The sera were diluted drop by drop in test tubes; the agglutination reaction was carried out on a matt surface; observations were made under a loupe under good illumination.

EXPERIMENTAL RESULTS

In the erythrocytes of all 13 fetuses investigated and in 12 newborn infants the agglutinogen Le(a+) was found. The agglutinogen Le(b+) was never found. The agglutination of the erythrocytes showed up to various extents: sometimes it could be distinguished under the loupe or even with the naked eye, but sometimes only under the microscope.

The discovery in the erythrocytes of fetuses and newborn infants of the Le(a+) agglutinogen represents an important link in the study of the Lewis system in man, particularly when we take into account that this agglutinogen is reported to exist in 80% of three-month infants, and that it is not until two years of age that the frequency of occurrence of agglutinogen Le(a+) attains the value of 20% found in adults. Le(b+) in the blood of young children is found less commonly than in adults, and not until the sixth year does the incidence rise to the value of about 70% characteristic of adults.

In determining the extent to which the agglutinogens of the ABO system were excreted, in six newborn infants it was shown that in the saliva of three of them which were of group O only agglutinogen H was present, in the saliva of one newborn infant agglutinogens A and H were present (A in the blood); in the saliva of another agglutinogens A, B, and H were present (A and B in blood). In one newborn infant agglutinogens A, B, and H were not shown to be present, and the blood contained agglutinogen A.

Thus, five of the newborn infants belonged to the category of secretors and one was a nonsecretor. It should be noted that in the erythrocytes of newborn secretors the agglutinogen Le(a+) was present; this is out of line with the relationship between the antigens of the Lewis system and the secretion of antigens of the ABO system characteristic of the adult.

The blood of 34 parents (we were unable to obtain the blood of three of the fathers) belonged to group Le(a-b+), five to group Le(a+b-) and eight to group Le(a-b-). All persons having blood of group Le(a-b+) were secretors of the agglutinogens of the ABO system, while those with blood of group Le(a+b-) were weak secretors or nonsecretors; five persons with blood of group Le(a-b-) were secretors, and three were nonsecretors.

The agglutinogen Le(a+) was present in the erythrocytes of fetuses and newborn infants resulting from the unions:

$$\begin{aligned}
&\text{Le}\ (a+b-) \times \text{Le}\ (a+b-)\\
&\text{Le}\ (a-b+) \times \text{Le}\ (a-b+)\\
&\text{Le}\ (a+b-) \times \text{Le}\ (a-b+)\\
&\text{Le}\ (a-b+) \times \text{Le}\ (a-b-)\\
&\text{Le}\ (a-b-) \times \text{Le}\ (a-b-)
\end{aligned}$$

i.e. for any arrangement of the parental Lewis system groups.

SUMMARY

The agglutinogen Le(a+) was demonstrated in the erythrocytes of 13 features spontaneously aborted on the 17-30th week of pregnancy, and of 12 newborn infants. The agglutinogen was present in these erythrocytes for all parental combinations of the Lewis system. The extent of secretion of the ABO agglutinogens was determined from the saliva of 47 parents and 6 newborn infants. Five of the infants were secretors and one was a nonsecretor. The presence in the erythrocytes of the newborn secretors of the Le(a+) agglutinogen was not in line with the relation characteristic of the adult system between the antigens of the Lewis and the ABO antigens. Thirty four parents with blood of the group Le(a-b+) were secretors, five which were Le(a+b-) were weak secretors or nonsecretors. Five persons of the blood group Le(a-b-) were secretors, and three were nonsecretors.

LITERATURE CITED

1. L. K. Arzhelas, Sud. med. ékspert. (1962), No. 4, p. 37.
2. O. Brendemoen, Acta path. microbiol. scand. (1961), V. 52, p. 55.
3. K. Jordal, Ibid. (1956), V. 39, p. 399.
4. R. Race and R. Sanger, Blood Groups in Man. Springfield (1958).
5. R. Rosenfield and G. Ohno, Quoted by E. Miller et al., Am. J. Phys. Anthropol. (1954), 12, p. 427.
6. W. Scheffler, Folia haemat. (Lpz.), (1959), Bd. 76, s. 232.
7. I. Unger, Quoted by R. Race and R. Sanger, Blood Groups in Man, Springfield (1958).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
