Changes in the Group A Antigen in a Case of Acute Myeloblastic Leukemia

By ADELA BARTOVA, Z. NOVOTNY AND J. SLEPIČKA

UNTIL RECENTLY, the immutability of erythrocyte antigens during the lifetime of the individual was regarded as undisputed. In the last few years, however, several reports have appeared introducing a new and, to a certain extent, contradictory point of view. The first of these reports dealt with the weak A quality of the red cells of a patient with acute leukemia who was known previously to be of group A. It was not possible to explain this finding genetically, and it was considered to be due to a change of the patient’s blood group during the course of his illness. Later Stratton described a patient who exhibited a uniform population of blood cells with a very weak A quality. Gold et al. then reported a patient with a double population of red cells, A1 and weak A. The percentage of A1 cells varied with the clinical and laboratory status of the illness. Salmon et al. reported a similar patient who had a mixture of A1 and 0 blood cells. In another case, described by these authors, a mixture of A1 and weak A cells were found. Here Salmon et al. pointed out that the weak A types described in previous publications were often found in patients with acute leukemia and they concluded that this change might be caused by the illness. All the preceding observations had been made in patients with acute leukemia as well.

We had the opportunity to observe a similar change of A antigen in a patient with acute leukemia.

Case Report

R. S., male, 36 years old. In 1957 he was treated at the 1st Medical Clinic in Olomouc for pleurisy, most probably actinomycotic, and for secondary anemia. Peripheral blood on admission: hemoglobin 57 per cent, red cells 2,680,000 leukocytes 11,000, (band forms 21 per cent, polymorphonuclears 54 per cent, eosinophils 1 per cent, monocytes 6 per cent, lymphocytes 18 per cent), platelets 187,000. Blood group A, Rh positive, Coombs’ direct negative, antibodies negative. The patient received a transfusion of 250 ml of A, Rh positive without reaction. After three months of antibiotic treatment he was dismissed with a normal peripheral blood (fig. 1.).

In June 1959, he had acute tonsillitis. In July petechiae appeared on his legs, he lost weight, had no appetite and felt very tired. In August 1959 he was hospitalized again. Physical findings: slightly icteric sclerae and dullness to percussion over the right lung base. The liver edge was palpable 3 cm. below the right costal margin and the spleen was not palpable. There were small petechiae on the skin of legs and arms. The hemo-
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Fig. 1.—Effect of therapy on hematologic and isoserologic findings.

globin was 56 per cent, red blood cells 2,800,000, white blood cells 24,000 with para-
myeloblasts 88 per cent, polymorphonuclears 2 per cent, eosinophils 1 per cent, mono-
cytes 1 per cent and lymphocytes 8 per cent. Platelets were 19,600. Urinalysis showed
no abnormality. Serum bilirubin was 1.2 mg. per cent, serum protein electrophoresis was
normal and the Coombs' test was negative. The bone marrow examination by Dr. B.
Wiedermann was typical of acute leukemia and showed the following features:

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Globin</td>
<td>56</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>2,800,000</td>
</tr>
<tr>
<td>White blood cells</td>
<td>24,000</td>
</tr>
<tr>
<td>Para-myeloblasts</td>
<td>88</td>
</tr>
<tr>
<td>Polymorphonuclears</td>
<td>2</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8</td>
</tr>
<tr>
<td>Platelets</td>
<td>19,600</td>
</tr>
<tr>
<td>Serum bilirubin</td>
<td>1.2 mg/100mL</td>
</tr>
<tr>
<td>Serum protein electrophoresis</td>
<td>Normal</td>
</tr>
<tr>
<td>Coombs' test</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Myeloblasts and promyelocytes of unequal size, some of them with vacuolated plasma.
Among these cells there were some with atypical forms of nuclei and nucleoli (paramyelo-
blasts and promyeloblasts). The nucleated red cells showed only few abnormalities. The
PAS stain was negative. Blood group determination showed only a very weak A quality.
In the next sample, examined two days later, no weak A quality was found. Anti-B ag-
glutinin was present in the serum. On the basis of this examination the patient was given
a transfusion of group O blood with a low titer of iso-agglutinins and was treated with
6-mercaptopurine and prednisone. In the course of treatment the number of white cells
and blasts in the peripheral blood diminished and the platelet count rose (fig. 1). The
patient was observed for five months. A very good clinical and hematologic improvement,
lasting nearly two months, was obtained in the course of his illness. The second remission
was shorter and incomplete. Following this the patient deteriorated and died eight
months after the first symptoms of leukemia.
RESULTS OF SEROLOGIC EXAMINATION

The first detailed examination was performed with sample No. 3. At the first routine examination we obtained the result 0 beta, Rh +, secretor A, non-secretor B and H. We did not succeed in proving the A quality in the cells, either by using the "Sanguitest" group O, B, or with serum from a group O immunized individual. Neither treating the cells with trypsin nor the use of acacia gum changed the result. We succeeded in proving the presence of the A quality in the cells only by absorption test. The patient's cells, in the proportion of one part of Sanguitest B serum to one part of cell-sediment absorbed the serum only slightly, though in comparison to the control cells, definitely. Remarkable was the fact that the cells absorbed the Sanguitest anti-A, as well, and that they absorbed it to a greater extent than did the A2 cells and approximately to the same extent as control A, cells in 3 per cent concentration (table 1).

In order to determine the percentage of A cells in the mixture, a modified method of differential hemolysis was used. The results indicated the presence of A cells 3 per cent and "non-A" cells 97 per cent. "Non-A" is used rather than "O" because the cells of the patient were not agglutinated by the anti-H\textsubscript{m} serum and they did not absorb it (table 2).

Following the repeated incubations and centrifugations of 10 ml. of the cell suspension with excess anti-A\textsubscript{m} or anti-A\textsubscript{n}, it was possible to separate A cells from the mixture. Anti-B\textsubscript{n} serum used as a control gave a negative result, as did anti-B\textsubscript{m} serum with the cells remaining after the differential hemolysis. These results prove that sample no. 3 contains, besides "non-A" cells, a small quantity of A cells which react as A1, but which could not be detected because of their small quantity.

Beta agglutinin in a titer of 1:64 was found in the serum, i.e., in a slightly lower concentration than the average found in healthy blood donors.

The patient was not a twin so that a chimera of embryonic origin could be excluded. Both his parents were group A1, so his pedigree did not necessarily predetermine the group A1, as had been stated two years ago (fig. 2).
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Fig. 2.—Family tree of the patient R. S.

Serologic examination three weeks later indicated no substantial change. The number of A1 cells detected by the method of differential hemolysis was now less than 2 per cent.

Three weeks later there was a very surprising change (sample no. 5). Even by the tube method, cells of group A were obviously present. By differential hemolysis 25 per cent A1 cells were found. The remainder of the nonhemolyzed cells were examined with an immune serum of the A group. By using differential centrifugation, we succeeded in separating a small quantity of agglutinable nonhemolyzed cells from this serum. With the hemolysin used, A2 cells were hemolyzed more weakly than cells of the patient. Sample no. 6 contained 60 per cent A cells. The cells absorbed not only anti-A11 serum but also anti-A11 serum, more strongly than did A2 cells, but more weakly than one-fifth the quantity of A1 cells. In sample no. 7 there were 70 per cent of A cells. A part of the remaining nonhemolyzed 30 per cent was agglutinable again. In sample no. 8, the percentage of A cells was determined by several methods: by differential agglutination with the strong B serum, 61 per cent, by differential agglutination with anti-A11 serum, 44 per cent. The cells which did not react on methods used were further differentiated (table 3).

The cells remaining after hemolysis contained single small agglutinates. The hemolysis did not increase after the addition of fresh hemolysin. After washing in isotonic saline, the agglutinates disappeared, but they reappeared after the addition of AB serum, which indicated that a small quantity of cells was still sensitized by an incomplete anti-A1 antibody. This could also be shown by agglutination in gum acacia.11 Sample no. 8 contained a "non-A" blood population and an A population which was evidently not homogenous. About two-thirds of this A population consisted of the strongly agglutinable A1 cells and the remaining one-third could be demonstrated by a strong anti-A serum
and partly by a strong hemolysin, as well. A simple mixture of control cells A₂ and O gave the same results with all methods. Figure 3 presents a schematic illustration of the relations found both in the sample no. 8 and the check mixture. From this we can conclude that in the sample no. 8 (as well as in the other samples), besides the cells A₂ and cells "non-A", there were red cells, the A quality of which had been weakened to a different extent.

The downhill course of the illness required a great number of transfusions and further isoserologic examinations were discontinued.

**Discussion**

From the former reports and from our own observation, it is evident that in some cases of acute leukemia, notably in patients of group A, a weakening of the A quality in the course of the illness can occur. This change goes over from the original A₂ to an A or to a quality which resembles that of group O, but which we prefer to call "non-A" (table 2). Both in one of the five formerly described cases⁵⁻¹⁴ and in our present case, the percentage of agglutinable

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**Table 3**

<table>
<thead>
<tr>
<th>The cells:</th>
<th>Reaction on serum:</th>
<th>Hemolysin</th>
<th>Serum B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonagglutinated by serum Anti-A₁₈₆</td>
<td>Anti-A₁₈₆</td>
<td>25%</td>
<td>27%</td>
</tr>
<tr>
<td>Nonhemolyzed by anti-A₁₈₆ hemolysin</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Nonagglutinated by serum B</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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**Fig. 3.**—Graph of the reaction of A₂ + O (control) red cells mixture and that of the reaction of the patient's red cells with serum anti-A₁₈₆, hemolysin and serum B.
cells varied in the course of the illness. Isoserologic findings of the described cases, incomplete as they may be, differ in details. In our own case, we noticed three kinds of cells in the last samples.

The question of the origin of these changes is very interesting. A chimera of embryonic origin is ruled out. The hypothesis that these changes are evoked by chemotherapeutic agents (similar to changes demonstrated by Moskowitz by the incubation of red cells in formalin) must also be rejected. The change in our and in Gold's case was noticed before therapy was begun; furthermore, during the course of therapy the original quality A or red cells partially reappeared. Salmon ascribed this change to an acquired somatic mutation, and localized the change to the bone marrow reticulum cells. A greater or smaller number of these cells can be involved in these mutations; in extreme cases, all the cells can be involved. According to this view, two populations of cells, or, in an extreme case, only modified cells, appear in the peripheral blood. Salmon accepts this explanation, although it is admittedly hypothetical. As our results demonstrated, especially in the examination of the last sample, we can differentiate the red cells of the patient into three types differing in their reactions with group specific agglutinins and hemolysins. If the hypothesis of somatic mutation is correct, we might suppose that this mutation occurred in more than one direction or degree.

Further, we may suppose that the change of group quality of red cells is related to chromosome abnormalities, both of number and form, which have been described in leukocytes in acute leukemia. Another possible explanation is that acute leukemia is accompanied by a change of metabolic processes in the hemopoietic tissue which could influence the changes of the antigenic properties of the red cells.

**Summary**

1. The authors describe a case of acute leukemia in a man of 36 years. During the course of his illness, a change in the agglutinability of the red cells was observed. The cells, originally A, changed continuously so that in some samples it was possible to detect as many as three populations of red cells, namely, A, weak A, and so-called non-A. In the course of the illness, fluctuations in the numbers of these cells in the whole population could be observed.

2. The clinical course and the results of detailed isoserologic examinations are presented.

3. Possible causes of these changes are discussed: (a) Somatic mutation; (b) chromosome abnormalities; (c) metabolic changes of the hemopoietic tissue.

**Summario in Interlingua**

1. Le autores describe un caso de leucemia acute in un homine de 36 annos de etate. Durante le curso de su maladia un alteration esseva observate in le agglutinabilitate de su erythrocytos. Le cellulas, que originalmente esseva del gruppo A, se alterava continuamente de manera que in certe specimens il esseva possibile deteger tres populationes de erythrocytos, i.e., A, debile A, e le si-appellate non-A. In le curso del maladia, fluctuationes in le numeros de iste cellulas intra le population total de cellulas esseva observabile.
2. Es presentate le curso clinic e le resultatos de detaliate examines iso-
serologic.
3. Es discutite le possibile causas de iste alterationes: (a) mutation somatic; 
(b) anormalitates del chromosomas; (3) alterationes in le metabolismo del 
tissu hematopoietic.

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