A B O Blood Type Alteration in Leukemia

—— Report of a Case and Review of Literatures ——

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A decade ago, it was universally believed that a man's ABO blood type was unchangeable for life. However, there are at least two exceptions of this rule: one is the "acquired red cell agglutinogen B" in cancer and the other the "loss of the red cell agglutinogens" in leukemia.[1)]

Recently we encountered a case of leukemia in whom the red cell agglutinogen A was lost. On April the 24th 1967, a blood sample of a 15-year-old girl patient with acute granulocytic leukemia was sent to our laboratory for blood-typing and cross-matching. Her red cells were inagglutinable with anti-A and anti-B serum, while her serum contained only anti-B agglutinin and no anti-A. This unusual findings which apparently was contrary to Landsteiner's rule*1 stimulated us to undertake a detailed serological examination of her blood and saliva. This paper reports this peculiar case, reviews the literatures and presents some speculation concerning the possible mechanisms of blood type alteration in leukemia.

CASE RECORD

The patient, a 15-year-old girl, had been entirely well until the beginning of March 1967, when she noticed petechiae on the legs, gingival bleeding and hypermenorrhea. She was admitted to Yamaguchi University Hospital on April the 18th, because of increasing lassitude, anemia and hemorrhagic diathesis.

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*1 According to Landsteiner's rule for the ABO groups, those isoagglutinins are regularly present in the plasma for which the corresponding agglutinogens are absent from the red cells, except during the neonatal period.

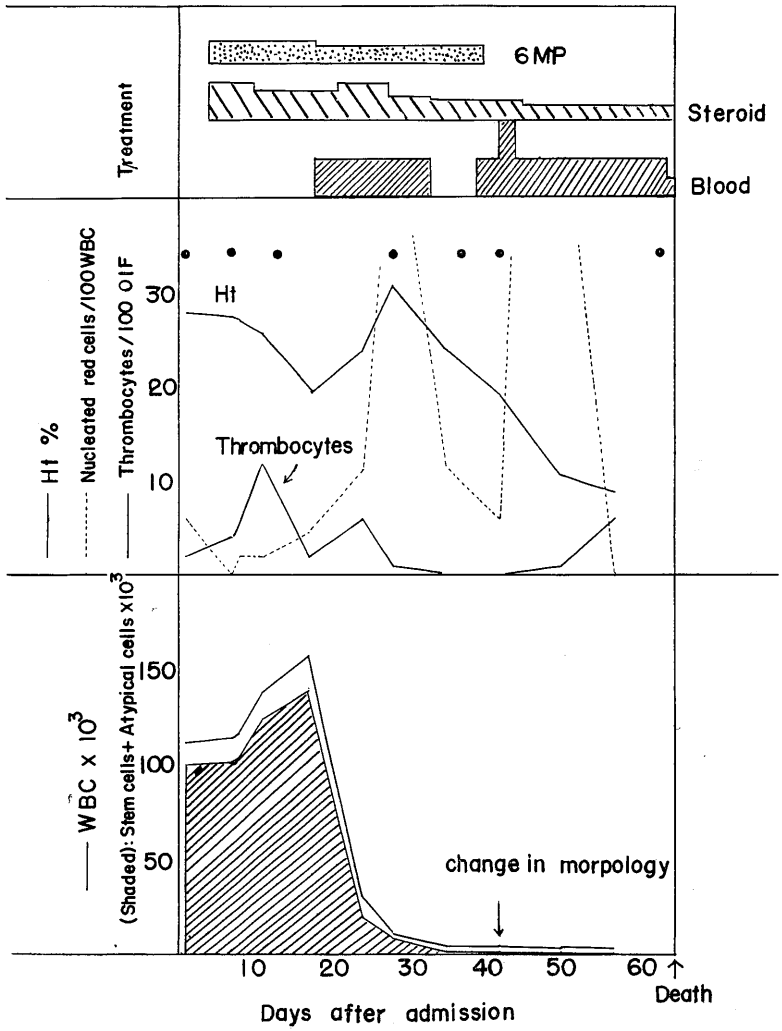


Fig. 1. Clinical course of the patient.
●: the days when blood samples were obtained.

On admission, abnormal findings on physical examination were confined to anemia and small areas of petechiae scattered over the whole body. Urinalysis is not remarkable except for a two plus urobilinogen, and fecal examination was two plus for occult blood. Hemoglobin concentration was 8.2g/dl, serum albumin slightly decreased and cholinesterase activity moderately decreased.
WBC was 112,200 with 90 % "stem cells" and "atypical young cells" which were strongly positive for peroxidase stain and negative for alkaline phosphatase. Normocytic normochromic anemia (moderate) and marked thrombocytopenia were noted. Sternal puncture showed hyperplastic marrow composed of 71.6%

"stem cells" and 3.3% "atypical cells". The M/E ratio was 9.2 : 1. Chromosome study of the bone marrow cells was not remarkable. There was no defect of plasma blood clotting factors. The diagnosis of stem cell leukemia (granulocytic) was made, and administration of dexamethasone and 6-mercaptopurine was started. Menstruation began to aggravate her anemia, and type A fresh blood transfusions were given. About a month later WBC returned to the normal range, but the thrombocyte count was consistently at almost zoro (per 100 oil immersion field). Bleeding tendency recurred, the patient became progressively more anemic in spite of blood transfusion, and died of severe undernutrition on June the 20th (Fig. 1). About 3 weeks before death, "atypical cells" having a strong resemblance to cells of the monocytic series appeared, suggesting transformation into Naegeli type monocytic leukemia. At times the number of nucleated red cells exceeded the white cells in the peripheral blood. Autopsy was not done.

SEROLOGICAL EXAMINATION

Most tests were carried out in two laboratories, the Clinical Laboratories of Yamaguchi University Hospital and the Clinical Laboratories of Atomic Bomb Casualty Commission (ABCC) Hiroshima, using somewhat different techniques. The first serological examinations were done on the blood and saliva taken before starting specific treatment of the leukemia.

Table 1. Agglutinability of the patient's red cells against various anti-sera is compared with that of "normal" type O, A and B cells.
V: no or variable agglutination by several type O serums
?: very weak agglutination with only one red cell sample out of a number of type A bloods
*: red cells from her parents and a healthy donor

Red Cells \ Sera	Anti-A	Anti-A_1	Anti-B	Anti-H (Ulex)	Anti-H (Eel)	Anti-O (Hen)	Type O	Patient's
O	−	−	−	+++	+++	++	−	−
A_1*	+++	+++	−	±～++	−～±	±～+++	+++	?
B	−		+++					++
Patient's	−	−	−	±	−	++	V	−

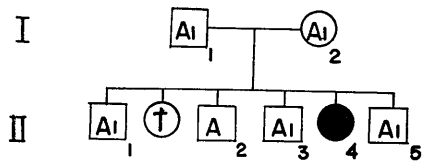
1) Examination of red cells: Her red cells were completely inagglutinable with any commercial anti-A*2, Anti-A_1 (Dolichos biflorus extract)*3 and many

*2 One manufactured by Dade Reagents (Anti-A agglutinin titer 1 : 4096) and several manufactured by Hiroshima Blood Bank (Over 1 : 256).

*3 One manufactured by Dade Reagents.

type B and A sera (Agglutination was determined by tube method after centrifugation at 1,000 r.p.m. for a minute, or by slide method after constant swirling for over 30 minutes). Saline, serum, bovine albumin, bromellin and indirect Coombs' methods consistently yielded negative results (Table 1). Five type O sera did not agglutinate the patient's red cells. However, only 7 out of 150 randomly taken sera agglutinated her red cells to varying degree but mixed-field agglutination was not seen. These seven sera were all type Owith anti-A saline agglutinin titers over 1 : 128. Indirect Coombs' tests confirmed these agglutinations as antigen-antibody reactions. Her red cells were agglutinated only feebly by anti-H (extract of Ulex europaeus)*4 in contrast to very strong agglutination of normal type O red cells. An eel serum (anti-H), which agglutinated normal type O red cells very strongly, failed to agglutinate her red cells. Agglutinability of the patient's red cells by anti-H seemed to be roughly equal to or somewhat weaker than usual type A_1 red cells (Table 1). Blood types other than ABO system are shown in table 2 together with other members of the family. No contradiction in parentage was noted.

Table 2. Blood typing of the patient (II-4) and the members of her family.



	A	B	A_1	C	D	E	c	e	M	N	S	s	P	K	k	Fy^a	Saliva
I—1	+	−	+	+	+	−	−	+	+	+	−	+	−	−	+	+	A Sec
I—2	+	−	+	−	+	+	+	−	+	+	−	+	−	−	+	+	A Sec
II—1	+	−	+	+	+	+	+	+	+	−	−	+	−	−	+	+	A Sec
II—2	+	−	+	+	+	+	+	+	+	−	−	+	−	−	+	+	A Sec
II—3	+	−	+	+	+	+	+	+	+	+	−	+	−	−	+	+	A Sec
II—4	−	−	−	+	+	+	+	+	+	+	−	+	−	−	+	+	A Sec
II—5	+	−	+	+	+	+	+	+	+	+	−	+	−	−	+	+	A Sec

In absorption-elution tests, one volume of normal type A, B and O sera were absorbed with one volume of the patient's red cells for 4hr at room temperature or overnight in ice-box, the red cells were washed 4 times with saline and the agglutinins were eluted into ½ volume of saline for 5 or 6 minute at 56°C.

*4 Anti-H Lectin (Purified) manufactured by Hyland Laboratories.

The absorbed serum agglutinated type B, A or both cells as strongly as non-absorbed serum. However, the eluate from her red cells exposed to type B serum agglutinated type A cells to the same degree as those from the normal type A cells. A surprising finding was that the eluate from her red cells exposed to type O serum agglutinated both type A and B cells very strongly (Table 3).

Table 3. Results of absorption elution tests. The patient's red cells absorb no significant amount of anti A detectable by the technic employed here. However they do eluate anti-A and socalled cross-reacting antibody.
※ indirect Cooms' test

Source of Agglutinin	Agglutination with Red Cells		
	A_1	B	O
Unabsorbed type O serum	+++ > 320	++ 80	− < 10
O after absorbtion by A_1 cells	− < 10	+ 40	− < 10
Eluate from A_1 cells	+ ++	+ W※	− −
O after abs. by patient's cells	+++ 160	++ 40	− < 10
Elu. from patient's cells	+++ +++	+++ +++	− −
Unabsorbed type B serum	160	< 10	< 10
B after abs. by A_1 cells	< 10	< 10	< 10
Elu. from A_1 cells	+	−	−
B after abs. by Patient's cells	160	< 10	< 10
Elu. from patient's cells	+	−	−
Unabsorbed typ A serum	− −	++ ++	− −
A after abs. by patient's cells	− < 10	++ 160	− < 10
Elu. from patient's cells	− −	− −	− −
Direct Elu. from patient's cells	−	−	−

The eluate from her cells exposed to type A serum did not agglutinate type B cells. These findings seemed to suggest that though the patient's red cells absorbed little amount of anti-A, they easily eluted the absorbed antibodies at 56°C, and that they eluted a much larger amount of so-called cross-reacting antibody, which was contained only in type O sera, than any of normal type A or B cells.*5 The red cells of her parents were not different from ordinary type A_1 cells in absorption and elution experiments.

2) Examination of serum : The patient's serum contained anti-B (Saline agglutinin titer 1 : 64) but not anti-A, tested with 40 fresh and 10 old (over 3

*5 Absorption and elution of agglutinins from type O serum reminded us the A_X type reported by Matsuda et al.[2)3)]

days) type A bloods and 150 randomly-taken bloods. However, we found one type A blood which was weakly agglutinated by her serum (saline agglutinin titier 1 : 4-8)*6 only when both the serum and the red cells were fresh. The donor was a healthy medical technologist. This peculiarity of the serum was shared with the patient's mother but no other members of the family. Otherwise no abnormal antibody was detected in her serum.

3) Examination of saliva : Blood group substances secreted in the saliva were detected by hemagglutination inhibition test. Type A substance was found in higher concentration in the patient's saliva than in the parents'. Type H substance was barely detectable in her saliva. All members of her family were type A_1 secretors.

In short, the red cells of this patient were indistinguishable from type O cells by routine agglutination tests with anti-A and anti-B. But the patient secreted type-A substance in her saliva, and there probably was no anti-A in her serum. Agglutination test with anti-H and absorption-elution experiments with type B and O serum showed that the blood type of her red cells was not O but "A-like".

From a two-year-old record (preserved in Kayoi Chū-gakkō), we found that her blood had been typed as A without difficulty. Therefore we concluded that the red cell type of our patient had changed from "normal" A to a variant of "weak A" presumably as a result of the disease.

COURSE AFTER TRANSFUSION

Deterioration of the patient's condition required blood transfusion before thorough examinations of her blood type could be completed. Fresh type A blood, 100ml daily, was carefully transfused without notable side-reactions. Blood samples were taken as indicated in fig. 1 to pursue the fate of the transfused type A red cells.

Twelve days after the first transfusion, about a half of the red cells in her circulation were agglutinated by anti-A. The red cells were completely agglutinated by (anti-A + anti-N). About one fifth of them were not agglutinated by anti-N only.

From a blood sample obtained 5 days after concluding a series of transfusions totaling 1,600ml type A blood over a period of 16 days, those red cells which

*6 To 0.1ml of the serially diluted serum was added 0.1ml of the 2% red cells in saline, the mixture was incubated at 37°C for 30 minutes, and agglutination was read with naked eyes after light centrifugation. Preliminary incubation of the serum at 56°C for 30 min. had no effect.

were agglutinable by anti-A (A (+) cells) and those not (A (−) cells) were successfully separated.*7 A(−) cells (30−40 % of all cells) were completely agglutinated by anti-N. There were differences in Hb F content and enzyme activities between A(+) and A(−) cells (see below). Four days later her red cells were about 60% A(+), and A(−) cells were completely agglutinated both by anti-M and anti-N. A(−) cells were partially agglutinated by some high-titered type O sera when the mixtures were swirled as long as a half to an hour at room temperature.

Resumption of blood transfusion did not prevent increasing seveirty of bleeding tendency and anemia. Two days before death, almost 90 % of her red cells were A(+).

Direct Coombs' test on her red cells (her own and transfused) was consistently negative on repeated testing. Her blood was compatible with all (except only one mentioned above) fresh type A bloods by major as well as minor cross-matching tests (bromellin method).

The fact that A(−) cells in her circulation were all N(+), namely consistent with her own red cells, suggested that the transfused A(+) N(−) cells did not lose A agglutinogen. In other words, we could found no evidence of blood type change of transfused cells from A(+) to A(−) in her circulation.

Whether her own red cells reverted to A(+) could not be acertained because of transfusion of type A bloods.

The patient did not produce anti-A antibody against transfused type A bloods.

RED CELL ALTERATION OTHER THAN BLOOD TYPE

What had happened to the patient's red cells aside from the blood type alteration we could not examine in detail for want of enough blood samples. Hemoglobin study showed no abnormality except a slight elevation in Hb F content, 1.8 % by Betke-Kleihauer method (normal value : less than 1.0%) which was distributed quite unequally among the red cells just as was the case for a normal adult and most of "symptomatic" high F conditions. After blood transfusion, Hb F content of A(−) cells was slightly but significantly higher than A(+) cells, and differences in some enzyme activities were also noted between the two types of red cells : leucine aminopeptidase 20 for A(+) and 10

*7 One volume of packed cell was suspended in 3 volumes of type B or commercial anti-A serum for 30 minutes at room temperature with constant aggitation. The mixture was lightly centrifuged (500 r.p.m. for 3 minutes) and the cells contained in the upper⅔ portion were collected. The procedure was repeated six times and A(−) cells were prepared. While the agglutinated masses were washed on absorbent cotten and A(+) cells were prepared.

Table 4. Some cases of A B O blood type change in leukemia. Only those cases in which red cell agglutinogen A was affected and sufficient data are available are listed here.

mix : mixed fieled agglutination, w : weak agglutination
↑ : increased agglutinability, or presence of A−H reciprocal relationship.
* : roughly equivalent to—cells α : anti-A, $\alpha\beta$: "cross-reacting" antibody or "anti−A+B"

Diagnosis	Original type	Characteristics of the altered red cells: Designation	Agglutination by α	Agglutination by $\alpha\beta$	Agglutination by −H	Absorption of α	Absorption of $\alpha\beta$	Elution of α	Elution of $\alpha\beta$	Agglutinins in serum α	Agglutinins in serum β	Saliva A	Saliva H	Remarks	
Myeloblastic	A_1	Ag	mix	w		A_1*		A_2*		−	64	−	−		Van Loghem et al 1957[4]
Acute	A		−	mix	↑	+		+		−	32	−	−		Stratton et al 1958[5]
Myeloid	A		mix	mix	w			+		−	+	+	+		Salmon et al 1959[7]
Myeloblastic	A		mix		↑	A_2*		+		−	+	−	−	Partial recovery with clinical remission	Gold et al 1959[8]
Myeloblastic	A	Ag	−		O*			−	+	−	+	+	+	Complete recovery to A_1 within 6 weeks	Hoogstraten et al 1961[11]
Eosinophilic	A_1B	A_2B A_2 B, O	mix	mix										Independent loss of A and B agglutinogens	Renton et al 1962[12]
Myeloblastic	A		mix		−	+					64	+		A_1−A−non A	Bartova et al 1962[13]
Chronic myeloid	A_2O		w		↑									Changes in Rh system	Tovey, et al 1961[14]
Chronic myeloid	AB		w							−	−			Recovery to A_1 B	Murakami et al 1967[21]
Acute myeloblastic	AB		−		O*			+		−	−	+		Multiple changes	Ayres et al 1967[16]
Acute granulocytic	A		−	no or w	A_1*	↓		+	+	?	64	+	±		Present case

units*8 for A(−) cells, lactic dehydrogenase 560 and 900, and acid phosphatase 27.6 and 19.7, respectively.

DISCUSSION

At least 25 instances of ABO blood type alteration in myeloid leukemia were reported[4)−21)] since 1957 when the first case was reported by van Loghem et al.[4)] Several such observations have recently been made in Japan,[21)] as well. Blood type alteration in leukemia may not be so rare as expected from the number reported in the past, though the frequency is unknown. No one has given a clear-cut interpretation of these curious phenomena. This case report is regretably unsatisfactory one. But judging from several reports (Table 4) we conclude as follows :

1. Red cell agglutinogens can be lost (or modified ?) in association with leukemia (esp. myeloid). Such phenomena have never been observed in other diseases.
2. These alterations do not affect the blood type substances secreted in the saliva.
3. The serum contains isoagglutinins accordong to the original blood type of the individual, and the patient does not produce antibodies against the lost agglutinogens.
4. Those red cells which have lost anti-A agglutinability absorb and elute anti-A and "cross-reacting" antibody.
5. Reactivities of the altered red cells differ remarkablly case by case. Two types may be discriminated, namely one in which loss of anti-A agglutinability of red cells is compensated by increase in anti-H agglutinability and one in which such compensation seems to be absent. Anti-A absorption capacity of the anti-A inagglutinable red cells vary from cases as strong as normal type A_1 cells to cases so weak as to be easily over-looked if elution tests are omitted.
6. Several serologically different red cells may coexist in the circulation of a single individual.
7. The lost agglutinogens may be restored, or reactivity of red cells may fluctuate.

The combination of anti-A inagglutinable red cells, absence of anti-A in the serum and presence of type A substance in the saliva has been named by Wiener

*8 Washed red cells were hemolysed by repeated freeze-thawing and diluted to 3.3mg Hb/dl. The enzyme activities were determined according to the routine methods for serum.[31)]

et al[22] as A_m type.*9 Summing up the serological data on A_m type*10 found in healthy donors reported by Weiner et al,[23] Dodd et al[34] and Salmon et al,[24] we feel that the difinition of A_m type should, in addition to the aforementioned 3 characteristics, include the forth characteristic that anti-H agglutinability of the red cells is increased in proportion to the decrease in anti-A reactivity. And the third characteristic should be qualified by the proviso "if the individual is a secretor" because A_m nonsecretor does not secrete type A substance in his saliva.

A_m red cells are not necessarily completely devoid of type A substance since they are inagglutinable by anti-A; they absorb and elute anti-A in various degree. They may be agglutinated by some type O sera, and even by high titered (immune) anti-A sera. Accordingly there is no definite criterion discriminating between A_m type[22)-24)34)] and von Loghem's[4] "A_g" type.*11 Similarly, some cases of "loss of red cell agglutinogen A" in leukemia may be impossible to differentiate from A_m type.

According to Weiner et al,[23] A_m type will be manifested when the rare recessive gene y, which modifies the development of the A antigen in the red cells, is active (yy homozygosity or absence of the dominant gene Y which enables gene A to operate). This hypothesis was affirmed by Salmon et al[24] in their observation of a family including 5 A_m type siblings for 2 generations.

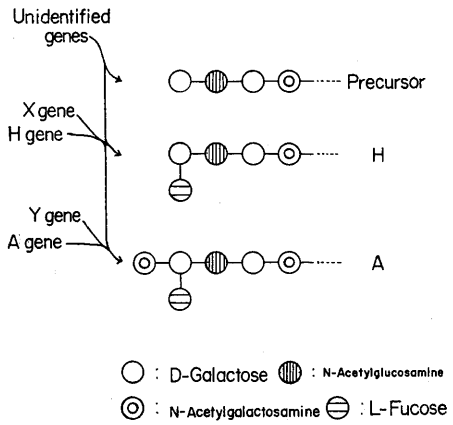


Fig. 2. Simplified Schematic representation of gene actions responsible for production of type A substance.[23)25)] H gene is completely suppressed by the absence of X gene or xx homozygosity (Bombay type), A gene by the absence of Y gene or yy homozygosity. Effect of yy homozygosity is incomplete and probably limited to antigens in the red cells (A_m type).

*9 A_m type of Wiener et al (the suffix m means monkey) was discovered in a patient with chronic myeloid leukemia. This might had been the first case of "loss of red cell agglutinogen A" in leukemia.

*10 The suffix m in their description means "modified".

*11 Van Loghem et al reported two curious bloods (one from the patient with myeloblastic leukemia who was known to have originally been type A, and another from a healthy blood donor) whose red cells were almost inagglutinable by anti-A, yet absorbed it in the same degree as usual type A_1 cells, and named their blood type as A_g.

In leukemia cases the patients have both A and Y gene (therefore not yy homozygous). Though a possibility that the disease may suppress the Y gene and thus inhibit the transformation of blood type specificity H on red cell into A cannot be denied, at present, many other factors should be taken into consideration: mutation of blood type genes (A gene, H gene, ...),[25] genes which activate or inactivate the blood type genes (Y gene, X gene, ...),[23] more primitive genes, and various environmental conditions which modify the blood type antigens, and so on (Fig. 2). If loss of red cell antigen A is not accompanied by increase in antigen H, participation of Y_y gene would be doubtful. In our case the red cells were not only inagglutinable by anti-A but also relatively incapable of absorbing it, yet they did not show any increase in anti-H agglutinability. However, some authorities regard such cases as incomplete loss of antigen A. More meticulous examination on this point is necessary. Moreover, blood type alteration in leukemia is not confined to A agglutinogen: alteration of B[17][20][21] and possibly H in ABO system, D.E. and c in Rh system[14][16] and I and i in Ii system[32] have been reported. The mechanisms of blood type alteration could not be explained by a single gene mutation or suppression of only particular one of the genes; it is more probable that gene mutations, if any, are multiple even in a given case.

Renton et al[12] reported a case which is very interesting in relation to mechanisms of the blood type alteration. A patient with eosinophilic leukemia (whose original blood type must be A_1B) had four different red cells in his circulation, namely type A_2, A_2B, B and O with the ratio 12, 26, 42 and 20 respectively. The findings were interpreted as that two sets of sequential changes, $A_1B \rightarrow A_2B \rightarrow B$ and $A_1B \rightarrow A_2B \rightarrow A_2$, had independently progressed. The transfused type A_1 red cells did not lose their anti-A agglutinability in the patient's circulation in their case as well as in our case. Renton et al supposed that various types of red cells had been built up from the beginning in the bone marrow because it would be improbable that, among the red cells which were uniformly circulating, some lost A agglutinogen exclusively or more than B and some B more than A. Salmon et al[26][27] also maintain without direct evidence a change in chromosome level. Another case which might contradict this assumption was observed by Hoogstraten et al.[11] Red cells (which were originally typed as A) of a patient with myeloblastic leukemia were completely anti-A inagglutinable and 6 weeks later reverted completely to usual A_1 type. Such a rapid and complete reversal could best be explained by assuming alteration of agglutinability of red cells already in circulation due to some environmental action. Alternatively all the anti-A inagglutinable red cells might have been lost from circulation during that period and completely replaced by newly formed type A_1 red cells.

In some cases of myeloid leukemia, do neoplastic "stem cells" give rise to not only myeloblasts but also monoblasts and rubriblasts? If so, red cells derived

from neoplastic cells may have different blood type specificities from the individual's own. Alternatively, does one pathogen not only provoke neoplastic transformation of myeloblast but also bring about certain abnormalities on the chromosomes of rubriblasts? In any case particularly interesting are the reports suggesting the presence of the Philadelphia chromosome in rubriblasts in chronic myeloid leukemia[33] and of other chromosome abnormalities in acute leukemia.[28] But how can the observation be explained that red cells restored the original A_1 characteristics synchronously with remarkable increase of neoplastic cells in peripheral blood at the terminal stage of illness?[11]

Are there any special humoral factors which alter the activity of the genes? Such an assumption may be convenient for explanation of the absence of "normal" red cells in our patient. Lowered antigenicity of several organs of leukemia patients could also be explained along these lines.[29] Were such humoral factors susceptible to experimental research, a blood type abnormality would serve as a convenient indicater.

Does myeloblastic leukemia create an unusual environment which favors proliferation as well as existence of some particular type of erythrocytic cells and is disadvantageous to the others? If so, certain erythrocytic cells having undergone a somatic mutation would rapidly increase in number and eventually exceed or even completely expel the "normal" red cells. We could not ascertain this possibility on our patient though the $T_{1/2}$ of Cr^{51}-labelled type A_1 red cells were only 2 days in her circulation, because extra blood loss from bleeding became uncontrolable at that time. In a case reported by Gold et al[8] survival of the transfused type A red cells was shorter than type O cells. His observation may be interpreted either as loss (or modification) of agglutinogen A on the red cells or as selective elimination of the type A cells from the circulation.

Are agglutinogens of the already mature red cells modified in leukemia? At first we were likely to assume the presence in her circulation of some enzyme activity which decomposed the blood type agglutinogen A. However, our effort to prove it was fruitless. Modification of the red cell agglutinogen due to the medical treatment[30] is not apparent in our case.

Thus, at present there is no convincing evidence on support of any of the many assumptions concerning the mechanisms of blood type alteration in leukemia. We feel that red cell alteration in leukemia should not be investigated merely from a blood serological point of view. Search for any alteration in genetically determined characters of red cells in leukemia will surely enable us to understand better leukemia itself.

SUMMARY

Blood type alteration in a patient with myeloid leukemia is reported. The

red cells of a patient, whose blood type had been A two years ago, was completely inagglutinable by several commercial anti-A and many type B sera, but they were variously agglutinated by some type O sera. They absorbed anti-A relatively little, but easily eluted anti-A and "cross reacting" agglutinin. They were agglutinated by anti-H as feebly as type A_1 red cells from her parents. Though it could not be acertained if anti-A was entirely absent in her serum, the patient did not produce antibody against transfused type A bloods. Her saliva contained type A substance in higher concentration than her parents'. Loss of agglutinogen A from the transfused red cells in her circulation could not be demonstrated. Literatures concerning similar cases are reviewed, and possible mechanisms of blood type alteration in leukemia are discussed.

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