Alteration of Blood Group Antigens in Leukemic Lymphocytes *

JEROME I. BRODY † AND LAWRENCE H. BEIZER

(*From the Department of Medicine, the Graduate Hospital, and the Division of Graduate Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa.*)

The purpose of this investigation was to delineate the presence of ABH blood group antigens on the surface of normal and leukemic lymphocytes and to determine whether this genetically directed and supposedly constant characteristic (1) is altered in the neoplastic cell. The study was an extension and synthesis of the following previously recorded observations. *1)* The recent demonstration that red cells and lymphocytes share common cellular antigens (2) implied this might also be the case for more specific antigenic determinants. *2)* Blood group substances are found not only in erythrocytes but are widely distributed in a multiplicity of body tissues and fluids (3) so that their suspected presence in the lymphocyte was credible. *3)* The observation that carcinomatous epithelia lose their blood group identity (4) suggested the possibility that a similar alteration might occur in the malignant lymphocyte.

The lymphocyte's tendency to clump spontaneously and its avidly adsorbing surface lead to interpretive difficulties when a number of immunologic methods, such as conventional agglutination, antibody consumption, and fluorescent labeling are used to define this cell's more exact antigenic structure. The technic (5, 6) of specific mixed cell agglutination (SMCA), as employed and described in this study, largely circumvents these problems since the only circumstance under which the lymphocyte may be linked to the indicator red cells of known blood group by type-specific anti-

body is when both cells share a particular, common type-specific antigen.

Methods

A. Patients

1) Leukemic group. There was a total of 11 patients with chronic lymphocytic leukemia, 8 males and 3 females, ranging in age from 41 to 77 years, from whom lymphocytes were obtained for the experimental procedures. Table I incorporates data pertinent to the study such as when the diagnosis of leukemia was made, the type of specific antileukemic treatment used in patient management, whether anemia was present, and the major lymphocyte forms observed in the peripheral blood.

2) Controls. The control subjects were purposefully divided into two categories. The first subclassification, designated base-line controls, comprised ten adult patients who either had or died from cardiovascular disease, were without any evidence of hematologic or immunologic abnormalities, and whose lymphocytes were used to quantitate base-line type-specific lymphocyte reactivity. The next subgroup was termed special controls because the nine patients in this class each had some inherent abnormality which might, by itself, modify the surface blood group antigens for the following reasons. It is conceivable that the somatic immaturity of three children, the aminopterin administered to one patient with psoriasis (7, 8), and the distorted immunity of two patients with disseminated lupus erythematosus might influence type-specific lymphocyte reactivity in an untoward fashion. Similarly, the lymphocytes from a patient with a disease of suspected but unproven viral etiology, such as infectious mononucleosis, in which morphologic changes simulating acute leukemia (9) are observed, or the cells from a patient with aplastic anemia in whom the bone marrow was functionally disturbed, might behave unlike normal lymphocytes in SMCA. Finally, the mixed agglutinability of the lymphoblasts from a 5-year-old child with acute lymphoblastic leukemia may differ significantly from the normal because of the marked cellular immaturity of these cells.

B. Blood typing

Ante- or post-mortem determination of all lymphocyte and epithelial cell donors' conventional ABO blood group was performed according to the standards of the American Association of Blood Banks (10) and included confirmatory back-typing with patient serum. Subtyping of the A antigen was not carried out.

TABLE I

Summary of data on the patients with chronic lymphocytic leukemia

Patient no.	Age	Time of diagnosis	Therapy	Anemia	Peripheral blood lymphocytes
	yrs				
1	41	September 1963	Triethylenemelamine* Chlorambucil	None	Mature type
2	65	November 1963	External X irradiation to mediastium	Moderate	Mature type
3	74	September 1961	Chlorambucil* Adrenal corticosteroids*	Severe	Mature type
4	77	Diagnosis just made	None	None	Mature type
5	58	May 1962	Chlorambucil	None	Mature type
6	70	1961†	Chlorambucil Adrenal corticosteroids External X irradiation	Moderate	75% mature type 15% prolymphocytes 10% smudge cells
7	69	Diagnosis just made	None	None	Mature type
8	47	January 1961	External X irradiation Adrenal corticosteroids* Chlorambucil*	Moderate	Mature type
9	57	August 1961	Triethylenemelamine P^{32} Chlorambucil	None	75% mature type 25% prolymphocytes
10	51	August 1960	Triethylenemelamine Chlorambucil	None	95% mature type 5% prolymphocytes
11	75	October 1956	Triethylenemelamine External X irradiation Chlorambucil	Moderate	Mature type

* Denotes patient receiving this treatment at time of study.
† Month unknown.

C. Patient secretor status

Saliva, collected from the first six leukemic patients, was immersed in boiling water for 10 minutes to destroy the enzymes that would otherwise inactivate group specific substances. After centrifugation to remove mucus and debris, equal volumes of saliva and type-specific antibody were incubated together for 10 minutes before the addition of 1 vol of the appropriate red cell suspension. The mixture was rotated manually for 6 minutes and agglutination recorded macroscopically and under dark field microscopy. Hemagglutination in this instance was compared to that induced with full strength and saline diluted antibody.

D. Serological reactants

1) Lymphocytes. Peripheral blood lymphocytes were obtained by defibrinating 40 ml blood in a 125-ml Erlenmeyer flask containing a glass bead for each milliliter of blood. The defibrinated blood was transferred to 40-ml plastic round-bottom test tubes and mixed with an equal volume of a 3% dextran solution, the dextran having a molecular weight of 188,000.[1] After incubation in a 37° C water bath for 45 minutes the blood-dextran mixture was

centrifuged at 90 g in 15- X 120-mm test tubes [2] for 30 minutes. The supernatant of the control blood contained, depending on the differential leukocyte count, 70 to 75% lymphocytes, 5 to 15% polymorphonuclear leukocytes, and 15 to 20% red cells, and that of the leukemic blood contained 90 to 95% lymphocytes. After centrifuging this supernatant at 2,300 g for 10 minutes, the second supernatant was discarded and the remaining sediment washed 4 times and made up to a 2% lymphocyte suspension with phosphate buffered saline, pH 7.4. Lymph node lymphocytes were harvested from mesenteric lymph nodes obtained in the autopsy room. The nodes were cut into sections 1 to 2 mm wide. These pieces were suspended and agitated in saline to dislodge the lymphocytes. To remove debris, undesired mesenchymal elements, and to homogeneously disperse the lymphocytes the cellular suspension was passed, sequentially, through surgical gauze, filter paper used in commercial coffee urns,[3] and two gauges (no. 40 and no. 105) of wire screen mesh.[4] The cells were washed, and a 2% suspension was made as described above.

[1] Pharmachem Corp., Bethlehem, Pa.

[2] This and all subsequent glassware was siliconized with Siliclad, Clay Adams, Inc., New York, N. Y.

[3] Filtrator Coffee Appliance Co., New York, N. Y.

[4] Tyler Woven Wire Screen Co., Cleveland, Ohio.

2) Epithelial cells. Epithelial cells, secured by abrading oral, buccal, and nasopharyngeal mucosae of healthy hospital personnel, were washed and suspended in saline as outlined for the lymphocytes.

3) Erythrocytes. The indicator red cells were prepared as a washed 1% suspension of erythrocytes pooled according to A, B, and O blood type and were collected from rotating volunteer donors or surgical patients whose transfusion requirements were being anticipated before operation.

4) Reagents used as antibodies. Commercial [5] anti-A and anti-B, each with a 4+ hemagglutination titer of 1:256, served as the isoagglutinins in the serologic procedures. The anti-H lectin, paired with cells only from type O patients [6] was an aqueous extract of *Ulex europaeus* seeds and demonstrated 4+ red cell agglutination up to a 1:32 dilution. Initially, the isoagglutinins and the lectin were employed in the assays over a broad titration range, the dilutions reaching 1:1,600 with the former antisera and 1:320 with the anti-H reagent. Ultimately, experimental evidence indicated that double dilutions of anti-A and anti-B up to 1:320 and of anti-H up to 1:64 were required to demonstrate optimal mixed agglutination and to quantitate lymphocyte reactivity. The number of the tube dilution indicates the potency relationship between the isoagglutinins and the lectin so that, for example, a 1:80 anti-A or anti-B dilution corresponds to an anti-H dilution of 1:16. These materials were stored in 0.5-ml samples at −20° C to maintain constant potencies.

E. Specific mixed cell agglutination assays

1) Prototype procedure. Although the mixed agglutination reaction has been shown to be a valid method to detect antigens of the ABH category in cells other than erythrocytes (5), a model system using epithelial cells, previously known to contain blood group antigens (12), was constructed to retest its applicability in this particular laboratory. Two-hundredths ml of a 2% suspension of epithelial cells obtained from a type A donor was added to each of 14 hemagglutination tubes arranged in two separate rows. To one row of tubes, 0.04 ml of parallel, serially diluted antibody, anti-A, was added to sensitize the cells, and to the other row discordant, anti-B antibody was added in a similar fashion. The reactants were incubated at 16° C for 1 hour and the epithelial cell agglutinates washed 3 times with 5 vol buffered saline. After transfer to clean test tubes the anti-A–sensitized cells then received 0.01 ml type A indicator red cells, and type B indicator erythrocytes were added to the epithelial cells sensitized with anti-B. The tubes again were incubated at 16° C for 2 hours after which wet coverslip preparations of the reactions were observed under dark-field for qualitative, mixed epithelial-red cell agglutination.

[5] Ortho Pharmaceutical Corp., Raritan, N. J.

[6] This combination does not fail to recognize that all erythrocytes and numerous nonhematopoietic elements contain the H antigen, but merely implies, as required in the experimental protocol, that type O cells carry it in greatest concentration (11).

2) Basic lymphocyte sensitization. Although the general scheme, proportions, and dilutions of reactants remained unchanged from the test for epithelial cell agglutinability, the performance of the mixed agglutination assay to detect blood group antigens of normal and leukemic lymphocytes differed somewhat from that outlined for the model system, mainly because it was crucially important to avoid confusing nonspecific antibody adsorption by the lymphocyte with a true antigen-antibody reaction between this cell and sensitizing antisera. Normal lymphocytes from A and B donors were each sensitized with serially diluted concordant and discordant antibodies. The clumped cells underwent multiple washings with 20 times their volume of saline. After each washing, 0.01 ml of indicator red cells, with type corresponding to the sensitizing antibody, was added to each lymphocyte-containing tube and, after requisite incubation, examined microscopically for mixed lymphocyte-red cell clumping. Nonimmunologically adsorbed antibody, as indicated by a tendency of discordant antibody-coated lymphocytes to form mixed agglutinates with appropriate indicator red cells, disappeared after the fourth washing, but the procedure was not terminated until the lymphocytes were washed a total of 7 times. The definitive A and B antigen assay, therefore, with both normal and leukemic lymphocytes, was performed as described above, but the consistent and predictable behavior of the cells after copious washing allowed the sensitization with discordant antibody and addition of red cells before the seventh wash to be omitted. Standardization of the anti-H agglutination was carried out by incubating type O red cells with lectin-sensitized lymphocytes, and because of the comparatively unstable bond created between *Ulex europaeus* extract and the H antigen (13), mixed lymphocyte-red cell agglutination no longer was recorded after the third wash. On this basis, therefore, two washings were considered sufficient to remove excess anti-H while leaving sufficient lectin for the actual H antigen titration. As a further control of the serologic method the reactivity of appropriate type normal lymphocytes was determined concurrently with every alternate assay of leukemic lymphocyte agglutinability.

Final reactivity of all assays was evaluated semiquantitatively so that when more than 75% of the visible indicator red cells were in the mixed aggregate, the result was graded +4; when 50 to 75% of the erythrocytes were clumped with the lymphocytes, the reaction was +3; when 25 to 50% of the red cells were attached to lymphocytes, the reaction was +2; and when 5 to 25% of indicator cells were seen in mixed agglutination, the reaction was read as +1. Mixed aggregates of autologous lymphocytes, contaminating red cells, and polymorphonuclear leukocytes, observed as isolated clumps upon initial sensitization, were eliminated with progressive washing and did not interfere with the interpretation of the agglutination titrations.

3) Reactivity of papain-treated leukemic lymphocytes. The decreased type-specific agglutinability of neoplastic lymphocytes, as observed early in the course of the study, suggested that their antigenic sites or antibody receptors

might not be exposed to react most favorably with the corresponding type-specific antibody. This possibility was examined by suspending the lymphocytes with a 1% papain [7] solution made in phosphate buffered saline to which was added an equal amount of 0.2% saline solution of L-cysteine hydrochloride.[8] The second reagent was freshly prepared for each experiment and served as enzyme activator (14). The mixture was incubated in a 37° C water bath for 10 minutes after which the lymphocytes were washed 3 times and treated as in the basic sensitization procedure.

F. Confirmation of reaction specificities

1) *Dispersal of agglutination.* Dispersion of agglutination is a well-known serological principle (15) which states that cells agglutinated by an antibody are liberated from formed aggregates upon incubation with a soluble antigen provided that the cell antigen originally combining with the antibody and the soluble antigen subsequently added to the agglutinates are identical. In the present experiments, boiled secretor saliva of known blood group, cleared by centrifugation and concentrated by dialysis, and saline as a control were added in 0.04-ml quantities to the completed mixed agglutination assays of parallel blood type. In addition, lyophilized porcine A- and equine B-isolated blood group substances,[9] each dissolved in a concentration of 4 mg per ml phosphate buffered saline, were used similarly to test the dispersal of agglutination phenomenon. The reactants were incubated at 16° C for 45 minutes after which the titrations were re-examined microscopically.

2) *Inhibition of agglutination by nonreducing monosaccharides.* With the idea in mind that substances with structures similar to or identical with the immunologically determinant group of a complex antigen are able to competitively prevent the reaction between an antigen of primary interest and its antibody (16) the carbohydrate known to be most specifically related to each individual blood group antigen (17) was incorporated in the type-specific agglutinations as follows. Solutions of the relevant carbohydrate, as 50 mg per ml saline, were substituted for the ordinary buffered saline so that the A, B, and O lymphocytes were incubated overnight at 16° C in dissolved N-acetylgalactosamine, D-galactose, and L-fucose, respectively. Type-specific sensitization was performed as before after which the cells were washed and indicator erythrocytes added to the antigen-antibody systems as for the basic lymphocyte type-specific assay.

Results

A. Patients as secretors

In all six instances patient saliva, when mixed with type-specific antibody, inhibited hemagglu-

---

[7] B. D. H. Reagents, Cambridge, Mass.

[8] Sigma Chemical Co., St. Louis, Mo.

[9] Supplied by Mr. William Pollack, Ortho Research Foundation, Raritan, N. J.
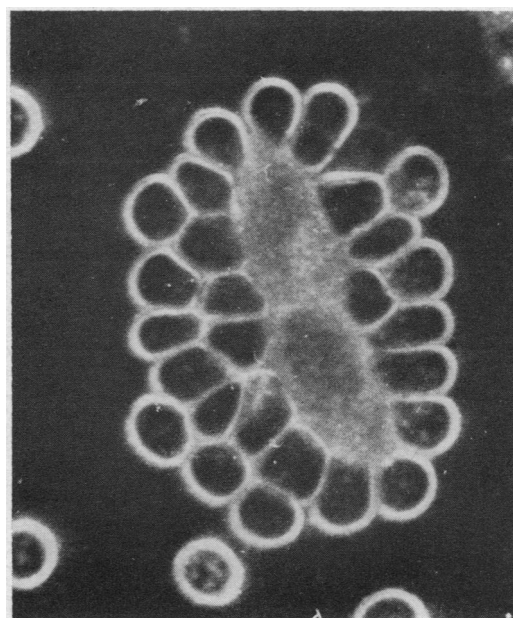

FIG. 1. MIXED AGGLUTINATION ASSAY WITH ANTI-A–COATED EPITHELIAL CELLS. Epithelial cell clumping with type A indicator red cells is evidence that both cells have the A antigen in common (× 900).

tination in terms of the size, cohesiveness, and stability of the red cell clumps formed. These reactions indicated the presence of secretor material inasmuch as the potency of the saline-diluted antibody appeared unaltered. The consistent results, unfortunately, discouraged further pursuit of this phase of the investigation.

B. Mixed agglutination assays

1) *Epithelial cells.* Although not evaluated quantitatively, mixed epithelial-red cell agglutination was observed in antibody dilutions up to 1 : 80 (Figure 1) with the anti-A–coated cells. The absence of mixed agglutination with anti-B–coated epithelial cells is evidence that nonspecifically adsorbed antibody may be removed, in this situation, by washing the epithelial cells three times. These results again indicate that the technique employed is valid for detecting type-specific antigens of the ABH category in cells other than erythrocytes.

2) *Lymphocytes.* The reactivity of peripheral blood and lymph node lymphocytes obtained from both groups of control patients is summarized in Tables II and III. Mixed agglutination of normal control lymphocytes (Figure 2) extended at least up to the fourth titration tube in nine instances,

TABLE II

Type-specific base-line control lymphocyte reactivity

Patient no.	Blood type	Lympho-cyte source*	Reac-tivity	Antibody dilution
1	A	PB	+1	1:80
2	O	LN	+2	1:4
3	A	PB	+3	1:40
4	A	LN	+1	1:40
5	O	LN	+2	1:8
6	A	LN	+2	1:40
7	A	LN	+1	1:80
8	B	LN	+1	1:40
9	A	LN	+2	1:80
10	B	LN	+1	1:40

* PB = peripheral blood, ante-mortem specimen; LN = lymph node, post-mortem specimen.

and only in one patient was the observed reactivity less than this (Table II). The lymphocyte response appeared to be independent of lymphocyte source or blood type. The results with the special control lymphocytes, in general, paralleled those of the other control group (Table III). However, two of the children (nos. 2 and 3) demonstrated slightly diminished reactivity as compared to normal, and the A antigen in the leukemic lymphoblast was not demonstrable beyond the 1:10 antibody dilution. In view of the fact that unequal antigen partitioning is a characteristic of type AB red cells, the anti-A titer of 1:10 most probably does not represent an abnormal decrease in the expression of the lymphoblast's A antigen (18).
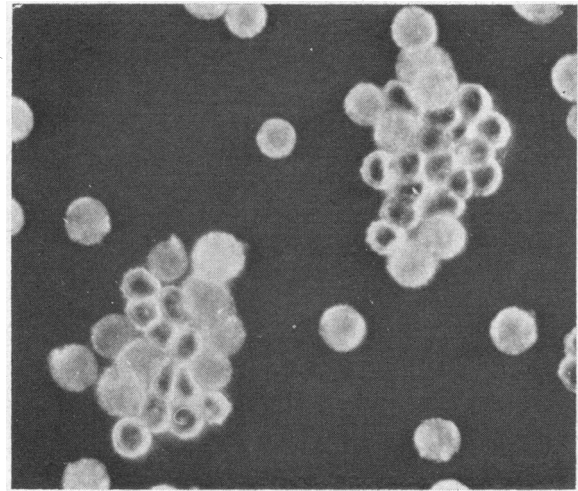

Fig. 2. REACTIVITY OF NORMAL, ANTIBODY-COATED LYMPHOCYTES IN SPECIFIC MIXED CELL AGGLUTINATION. Note absence of free floating red cells (× 450).

The mixed agglutination patterns observed with leukemic lymphocytes (Figure 3) were distinctly dissimilar from those occurring with both types of control cells. These agglutination reactions are summarized in Table IV. Reactivity was completely absent in the lymphocytes from five patients and only weakly detectable with full strength antibody in cells of a similar number of individuals. The one gross exception to the over-all scheme of response is the last patient (Table IV), whose cells

TABLE III

Type-specific special control lymphocyte reactivity

Patient no.	Age	Reason for inclusion	Blood type	Lymphocyte source	Reactivity	Antibody dilution
1	1 day	Somatic immaturity	B	LN	+3	1:40
2	10 weeks	Somatic immaturity	A	LN	+1	1:20
3	2 years	Somatic immaturity	O	LN	+1	1:4
4	30 years	Treatment with aminopterin	A	PB	+2	1:40
5	16 years	Disseminated lupus erythematosus	B	PB	+2	1:80
6	45 years	Disseminated lupus erythematosus	A	PB	+1	1:80
7	21 years	Infectious mononucleosis	A	PB	+2	1:80
8	55 years	Aplastic anemia	A	LN	+1	1:40
9	5 years	Acute lymphoblastic leukemia	AB	PB	+1 +1	1:10* 1:80†

* With anti-A.
† With anti-B.

reacted in a manner that was overtly divergent from that observed with all the other lymphocyte groups. In this instance mixed agglutination occurred with a 1:320 antibody dilution initially, and then 6 months later decreased reactivity was detectable with a maximal antiserum dilution of 1:20. Incubation of the leukemic lymphocytes with cysteine-activated papain did not visibly alter their agglutinability in any of the assays. As with their control relatives, the immunologic behavior of the leukemic lymphocytes appeared to be unrelated to the clinical variables included in Table I.

C. Confirmatory studies

1) Dispersal of agglutination. Addition of type-specific secretor saliva and isolated A and B blood group substances to all reactions in which specific mixed cell agglutination had been observed initially resulted in an appreciable change in their appearance when they were re-examined after this maneuver. Mixed agglutination either was markedly reduced, in the tubes with more concentrated antibody, or had disappeared completely where dilute antibody was present. This is evidence that the lymphocytes and indicator red cells shared identical blood group antigens with each other and with the added blood group substances. The conversion could not be induced with buffered saline.

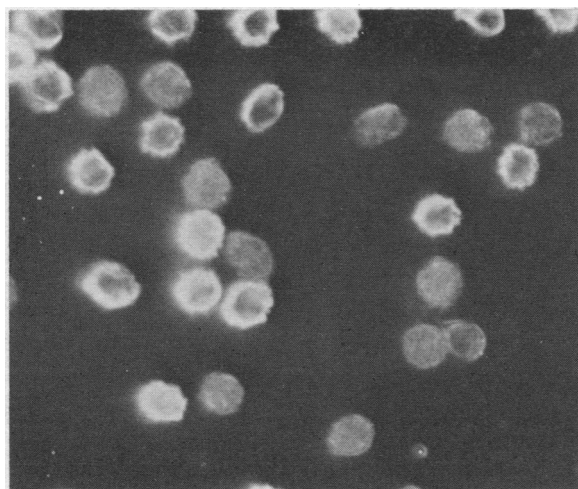*2) Carbohydrate inhibition.* Formation of mixed

FIG. 3. DECREASED TYPE-SPECIFIC REACTIVITY OF LEUKEMIC LYMPHOCYTES AS INDICATED BY THEIR FAILURE TO FORM MIXED AGGLUTINATES WITH THE BRIGHT-APPEARING INDICATOR RED CELLS (× 450).

TABLE IV

Type-specific leukemic lymphocyte reactivity

Patient no.	Blood type	Lympho-cyte source	Reac-tivity	Antibody dilution
1	B	PB	+1	Undiluted
2	B	LN	0	
3	O	LN	0	
4	O	PB	0	
5	A	PB	0	
6	A	PB	+1	Undiluted
7	B	PB	0	
8	A	PB	+1	Undiluted
9	A	PB	+1	Undiluted
10	B	PB	+1	Undiluted
11	A	PB	+2	1:320
		PB*	+2	1:20

* Performed 6 months after first titration.

agglutinates was completely prevented or appreciably diminished with lymphocytes that had been suspended in the sugars, strongly suggesting that the latter's presence in the reacting mixtures prevented antibody from combining with type-specific surface lymphocyte ABH antigens.

Discussion

There are two major observations that arise from this study. The first is that ABH blood group antigens are present and may be detected on the surface of normal human lymphocytes as determined by the specific mixed cell agglutination technic. The manner in which the mixed agglutination assays were performed, the disappearance of mixed clumping when blood group substances were added to the completed reactions, as in the method for dispersal of agglutination, and the inhibition of mixed agglutination when the nonreducing monosaccharides were interposed in the type-specific antigen-antibody combinations are supportive evidence that the recorded lymphocyte reactivity did not occur fortuitously. Of probably greater importance, however, is that these same antigens, expressed mainly as an absence or diminution of type-specific mixed agglutination reactivity, appear to be altered in the leukemic lymphocyte. It is difficult to explain this phenomenon since one of its few analogous precedents is alteration or change in conventional agglutinability of erythrocytes as reported in patients with several types of leukemia (19–28). In these cases the more persistent, but not the only, pattern has been general suppression of the A antigen or acquisition of the B antigen by type A red cells.

The presently described variations in leukemic lymphocyte reactivity may be considered in the light of what is currently known of the dynamics of agglutination reactions and the general origin, development, and expression of blood group antigens. Inasmuch as agglutination occurs only when the number and the stability of combinations between antibody molecules and antigen sites are sufficient to provide a firm linkage between the participating cells, the failure of mixed agglutination to take place may be due to certain externally induced deficiencies in the components of this reaction (29). These inadequacies may take the form of change in the spatial orientation and consequent inaccessibility of the lymphocyte's available antigenic sites (30), developing and poorly reactive heterogeneity of the macromolecules of the surface blood group specific substances (31), or a broad, perhaps enzyme-induced, destruction of the entire surface membrane of the leukemic cell (32). Somewhat against this hypothesis is the failure to relate the type-specific reactivity of both normal and leukemic lymphocytes to drug, radiomimetic, or radiation therapies, blood type, source, and form of lymphocyte, exposure to a proteolytic enzyme, and clinical or immunological state.

Another basis for the observed abnormality may be connected to the developmental growth of the leukemic lymphocyte. If the decreased expression of the A and A_2 red cell antigens in the fetus as compared to the adult (33, 34) is taken as an illustration in point, then another possible reason for the manner in which the leukemic lymphocyte reacted in SMCA is its origin from somatically immature lymphoid and hematopoietic tissues. This approach, however, is partially controverted by the fact that adult antigen reactivity generally may be approached during the first year of life and by the lymphocyte reactivity of special control Patients 2 and 3 (Table III) which, although somewhat diminished, still exceeded that observed with the lymphocytes from the patients with chronic lymphocytic leukemia.

An additional speculative theory is that the described serologic abnormalities may represent a fundamental defect in the evolution of the ABH antigens from their pristine substrate (30). Although this irregularity was reflected almost exclusively in a diminution of reactivity, the initial comparative hyperagglutinability of the lymphocytes of Patient 11 (Table IV) also may mirror disorganization of blood group substance precursors. Whether the apparent increase in antigen content or availability in this last case is singularly associated with the long duration of the disease is a moot question at present. Similarly, the factors initiating these alterations, which have their limited counterpart in a number of genetically determined erythrocyte blood group deletions (35) and of which Bombay blood is a prime example, remain undisclosed for the moment. If this newly described serologic abnormality is indeed genetically controlled and represents actual loss of a marker antigen, then the retention of secretor status by the first six leukemic patients tested for this trait suggests that the deletion effect may be limited only to lymphocytes and their progenitors. It is possible, however, that continued survey of all the patients with leukemia for secretor material might have helped to define more clearly the extent of this anomaly.

Finally, it is relevant to suggest that this decreased detectability of blood group antigens in the lymphocytes of chronic lymphocytic leukemia may, in some way, be related to the genesis of this form of hematologic malignancy and, by creating a potential state of loss of self-recognition, to the autoimmune phenomena that patients with this disease demonstrate (36, 37).

Summary

With the technic of specific mixed cell agglutination (SMCA) ABH blood group antigens were demonstrated on the surface of normal lymphocytes. These antigens are expressed in infancy and childhood, remain identifiable in patients with benign disorders of the lymphoreticular organs, and are not influenced by cytotoxic agents or certain abnormal immune states. Of greater interest, however, was that type-specific reactivity of the lymphocyte in chronic lymphocytic leukemia was either markedly decreased or completely absent as compared to the mixed agglutinability of its normal relative. Whether this newly described serologic abnormality represents an inability to detect these antigens or an actual loss of genetic markers remains unknown at present as does its relation to the genesis of this particular form of malignant blood dyscrasia.

References

1. Race, R. R., and R. Sanger. The inheritance of blood groups. Brit. med. Bull. 1959, 15, 99.
2. Brody, J. I., and L. H. Beizer. The cross-reactivity of lymphocyte and red cell antibodies. J. Lab. clin. Med. 1964, 63, 819.
3. Glynn, L. E., and E. J. Holborow. Distribution of blood-group substances in human tissues. Brit. med. Bull. 1959, 15, 150.
4. Kay, H. E. M. A and B antigens of normal and malignant cells. Brit. J. Cancer 1957, 11, 409.
5. Coombs, R. R. A., D. Bedford, and L. M. Rouillard. A and B blood group antigens on human epidermal cells. Demonstrated by mixed agglutination. Lancet 1956, 2, 461.
6. Brody, J. I., and L. H. Beizer. Globulin coating of neoplastic lymphocytes in chronic lymphocytic leukemia. Blood 1963, 22, 139.
7. Harrington, W. J., M. J. Halloran, V. G. Kirchoff, E. H. Reinhard, R. Grossman, and B. White. Some immunologic aspects of the evolution and treatment of leukemia. Trans. Ass. Amer. Phycns 1962, 75, 73.
8. Halloran, M. J., and W. J. Harrington. Coding of bacterial nucleic acid with alkylating agents. Clin. Res. 1963, 11, 232.
9. Downey, H., and C. A. McKinlay. Acute lymphadenosis compared with acute lymphatic leukemia. Arch. intern. Med. 1923, 32, 82.
10. Technical Methods and Procedures of the American Association Blood Banks, revised ed. Chicago, Ill., American Association of Blood Banks, 1960.
11. Stratton, F., and P. H. Renton. Practical Blood Grouping. Oxford, Blackwell Scientific Publications, 1958, p. 122.
12. Swinburne, L. M., B. B. Frank, and R. R. A. Coombs. The A antigen on the buccal epithelial cells of man. Vox Sang. (Basel) 1961, 6, 274.
13. Solomon, J. M. Quantitative studies of the reactions of Ulex europeus. Transfusion (Philad.) 1963, 3, 428.
14. Stratton, F. A slide test for the detection of Rh antibodies using papain treated red cells. Vox Sang. (Basel) 1953, 3, 43.
15. Lawler, S. D. Genetical study of Gm groups in human serum. Immunology 1960, 3, 90.
16. Landsteiner, K. Spezifische Serumreaktionen mit einfach zusammengesetzten Substanzen von bekannter Konstitution (organischen Säueren). XIV. Mitteilung über Antigene und serologische Spezifität. Biochem. Z. 1920, 104, 280.
17. Watkins, W. M., and W. T. J. Morgan. Inhibition by simple sugars of enzymes which decompose the blood-group substances. Nature (Lond.) 1955, 175, 676.
18. Watkins, W. M., and W. T. J. Morgan. The A and H character of the blood group substances secreted

19. by persons belonging to group A₂. Acta genet. (Basel) 1957, 6, 521.
19. Van Loghem, J. J., H. Dorfmeier, and M. van der Hart. Two A antigens with abnormal serologic properties. Vox Sang. (Basel) 1957, 2, 16.
20. Stratton, F., P. H. Renton, and J. A. Hancock. Red cell agglutinability affected by disease. Nature (Lond.) 1958, 181, 62.
21. Gold, E. R., G. H. Tovey, W. E. Benney, and F. J. W. Lewis. Changes in the group A antigen in a case of leukemia. Nature (Lond.) 1959, 183, 892.
22. Bhatia, H. M., and L. D. Sanghvi. A variant of blood group A in a leukemia patient. Indian J. med. Sci. 1960, 14, 534.
23. Crowley, L. V., and R. C. Munkittrick. ABO blood group alterations in leukemia. J. Canad. med. Ass. 1960, 83, 216.
24. Hoogstraten, B., R. E. Rosenfield, and L. R. Wasserman. Change of ABO blood type in a patient with leukemia. Transfusion (Philad.) 1961, 1, 32.
25. Bartova, A., Z. Novotny, and I. Slepicka. Changes in the group A antigen in a case of acute myeloblastic leukemia. Blood 1962, 19, 566.
26. Michaels, L., and E. McNamara. Loss of blood-group-B antigen in chronic lymphocytic leukemia. Lancet 1962, 2, 512.
27. Richards, A. G. Loss of blood-group-B antigen in chronic lymphatic leukemia. Lancet 1962, 2, 178.
28. Gold, E. R., and L. Hollander. Blood groups and leukemia. Blut 1963, 9, 188.
29. Goodman, H. S. I. Analysis of immune agglutination of red cells. Transfusion (Philad.) 1964, 2, 327.
30. Morgan, W. T. J. A contribution to human biochemical genetics; the chemical basis of blood group specificity. Proc. roy. Soc. B 1960, 151, 308.
31. Greenberg, C. L., D. H. Moore, and L. A. Nunn. Reaction of 7S and 19S components of immune rabbit antisera with human group A and AB red cells. Immunology 1963, 6, 121.
32. Watkins, W. M. Changes in the specificity of blood-group mucopolysaccharides induced by enzymes from Trichomonas foetus. Immunology 1962, 5, 245.
33. Constantoulakis, M., H. E. M. Kay, C. M. Giles, and D. Parkin. Observations on the A₂ gene and H antigen in foetal life. Brit. J. Haemat. 1963, 9, 63.
34. Solomon, J. M. Comparison of fetal and adult ABH antigens by the quantitative hemagglutination technique. Transfusion (Philad.) 1963, 3, 185.
35. Race, R. R. Editorial. Vox Sang. (Basel) 1957, 2, 2.
36. Brody, J. I., and S. C. Finch. Serum factors of acquired hemolytic anemia in leukemia and lymphoma. J. clin. Invest. 1961, 40, 181.
37. Brody, J. I. Reactivity of red cell eluates and serums in patients with acquired hemolytic anemia and chronic lymphocytic leukemia. J. clin. Invest. 1962, 41, 471.