Erythrocyte Mucoids in Acquired Autoimmune Hemolytic Anemia

By Carson H. Tishkoff

The precise mechanism of red cell injury in acquired autoimmune hemolytic anemia (AHA) has not been defined. Various concepts of red cell injury have been proposed and these have been reviewed critically by Dacie. The hypotheses have been emphasized: (1) Hemolysis is related to alteration of the red cell surface structures by certain injurious agents (virus, bacterial enzymes or other products, or chemicals) and (2) antibodies are formed against normal red cells as a result of an aberration of certain antibody-producing clones. Since both concepts imply that alterations in the red cell surface structure play a major role in AHA, either by protein-chemical or protein-protein interaction, it is reasonable to assume that changes in membrane composition may occur in patients with these disorders.

Yachnin and Gardner have compared the red cell mucoid composition in normal subjects and patients with various hemolytic anemias. Their findings, which were limited to the red cell sialic acid content, demonstrated an increase in mean corpuscular sialic acid apparently related directly to the degree of reticulocytosis. No attempt was made by these investigators to compare Coombs’ positive and negative hemolytic anemias, with the exception of paroxysmal nocturnal hemoglobinuria, with comparable reticulocytosis.

In this report, the erythrocyte mucoid composition has been compared in normal subjects and in patients with AHA. As suitable controls, patients with various types of Coombs’ negative hemolytic or blood-loss anemia with reticulocytosis were included. In addition, the relationship of serology, blood group phenotype, and leukocyte contamination to membrane mucoid composition was also investigated.

Methods

Clinical Material. Normal red cells were obtained from young males with no hematologic disorder. The control subjects comprised 11 individuals with various types of Coombs’ negative anemia. Two determinations were made on subjects Sp and Le from blood obtained on different days which resulted in a total of 13 determinations from this group. Subjects with AHA were selected on the basis of a strongly positive Coombs’ test, significant anemia and the presence of appreciable reticulocytosis. This group was composed of 6 patients with secondary hemolytic anemia (lymphatic leukemia, reticulum cell...
sarcoma and collagen disease) and one with idiopathic AHA. Three patients (Ma. Li, Co) had significant elevation of cold agglutinin titer and were classified as cold-type AHA; the remaining 4 had warm agglutinins. Several subjects with AHA were studied on 2 or 3 different occasions so that a total of 10 determinations were made for the entire group.

Complete blood counts were performed by usual methods. Reticulocytes were enumerated by the method of Brecher, employing new methylene blue. The direct and indirect Coombs tests, cold agglutinin titers, and autoagglutinations were determined by previously described methods. Hematologic data are briefly summarized in Tables 1 and 2.

Isolation of Red-Cell Stroma. Forty ml. of blood was withdrawn into a 50 ml. pyrex tube containing 1 ml. of heparin (Upjohn heparin, 1000 units/ml.). In severely anemic subjects, 80 ml. of blood was employed. Blood counts were obtained on the heparinized blood. This procedure was modified in subjects with WBC of 40,000 or greater by initially removing white cells by sedimentation with polyvinylpyrrolidone (PVP). The sedimented red cells were then restored to their original volume by addition of isotonic saline and blood counts were obtained. The blood was centrifuged in a refrigerated centrifuge at 1800 g at 4 C. for 35 minutes. The supernatant plasma and buffy coat were removed by gentle aspiration with a 20 ml. syringe. Four ml. aliquots of red cells were carefully washed in cold saline and the supernatant removed by gentle aspiration. The pipette was washed free of cells by repeated rinsing with cold isotonic saline and then each aliquot was diluted to approximately 45 ml. with cold saline solution. Each tube was stirred with a glass rod and centrifuged in the cold at 1800 g for 10 minutes. The supernatant was removed by gentle aspiration. The washing step was eliminated in some individuals with potent cold hemolysins since considerable hemolysis occurred during the washing procedure.

The stromal fraction was isolated from hypotonic hemolysed red cells by the method of Tishkoff and his associates essentially as described, except that only 2 washings of the stromal material were made.

Hydrolysis of Stromal Mucoids. Chemical analyses were limited to fucose, sialic acid and protein residue of the stromal mucoids. Svennerholm, Yachnin and Gardner, Warren and Eylar and his co-workers have demonstrated that the terminally positioned sialic acid is split quantitatively from glycoproteins by hydrolysis with dilute acid. Likewise, Winzler has demonstrated that fucose is split from glycoproteins under similar condition of mild acid hydrolysis. Although 100 per cent of the red-cell sialic acid can be recovered under these conditions, it is not known whether total fucose is split from the carbohydrate moiety by dilute acid hydrolysis. It is possible that fucose located within the mucoid structure or in a bridge position may not be rendered soluble.

Duplicate stromal preparations derived from 4 ml. aliquots of red cells were suspended in 5 ml. of 10 per cent (w/v) trichloracetic acid (TCA) and transferred quantitatively to a 15 ml. graduated conical centrifuge tube. The tubes were immersed in boiling water for 15 minutes. It was necessary to stir frequently with a glass rod to avoid severe bumping and to disperse the coagulated proteins. The tubes were cooled in running tap water and diluted to 12.5 ml. with distilled water. Both duplicate samples were then combined by filtering through a single high-retentive, low-nitrogen filter paper. The TCA filtrate from the combined preparations (approximately 25 ml.) was employed for fucose and sialic acid analyses.

TCA insoluble protein material was allowed to dry overnight on the filter paper. The paper was then carefully folded in a manner to retain the dried protein, stapled and defatted by consecutive extractions with alcohol-ether and chloroform-petroleum ether, as previously described. The defatted protein was then air-dried and digested for total nitrogen determination.

Isolation of Leukocytes. Lymphocytes and granulocytes essentially free of red cells were isolated from subjects with chronic lymphocytic and chronic granulocytic leukemia. Heparinized blood was sedimented in the presence of excess fibrinogen. The leukocyte-rich plasma was removed, centrifuged at 1800 g and the leukocyte pellet extracted with an equal volume of 10 per cent TCA in boiling water for 15 minutes. Chemical determinations were performed on the TCA filtrate.

Chemical Analyses: Fucose was assayed by the method of Dische and Shettles with L
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(--)-fucose as a reference standard. The duplicate variability was ±2.5 per cent. Control studies established the fucose to be stable under the conditions of hydrolysis. Sialic acid was determined by the thiorarbucic acid method of Warren\textsuperscript{11} employing a Beckman DU spectrophotometer. It was found necessary to correct for the presence of 2-deoxyribose in the TCA extracts derived from WBC contamination. This correction was made by obtaining absorbancy (O.D.) values of the chromophore at 549 nm and 532 nm and employing equation 2 of Warren. However, the constants of this equation were revised since the molar extinction coefficient of crystalline sialic acid at 549 nm of 68.000 was not identical to that observed by Warren. The following equation was used:

$$\mu\text{moles of sialic acid} = 0.076 \times \text{O.D.}_{549} - 0.033 \times \text{O.D.}_{532}$$

Conversion of micromoles to micrograms was obtained by multiplying by 309. A ratio of O.D.\textsubscript{549}/O.D.\textsubscript{532} of less than 2 indicated appreciable contamination with 2-deoxyribose derived from WBC nuclear material. The duplicate variability of the assay was ±1.5 per cent.

Qualitative identification of individual sugars in the TCA extracts was made by paper partition chromatographic methods. Solvents employed were tert-butyl alcohol, methyl ethyl ketone, formic acid, water (40:30:15:15) and n-butanol, acetic acid, water (50:12:25). The following monosaccharides were identified in TCA extracts of membranes prepared from reticulocyte-rich red cells free of leukocytes: fucose, ribose, sialic acid and traces of hexose.

Total stromal protein nitrogen (N) was determined by the Kjeldahl method. Protein was calculated from protein-N by employing a factor of 6.25.

RESULTS

Variation in Erythrocyte Mucoid Composition in Subjects with Different Anemias (Coombs' Negative). Table 1 shows the results of 13 determinations from 11 hospitalized patients with various hemolytic and blood-loss anemias. Analytical values are expressed on a weight basis of stroma derived from 1 ml. packed RBC. The ratios of sialic acid and fucose to stromal protein are also included, thereby minimizing errors resulting from variations in mean corpuscular volume incurred during washing procedure, enumeration of red cells, and loss of stromal constituents due to chemical manipulations. Protein content was greater than normal, ranging from 8.6 to 16 mg./ml. RBC, the mean being 11.2 mg./ml. RBC. This increase in mean protein value paralleled the reticulocyte count. The mean values of sialic acid and fucose were identical to the values observed in normal subjects, although considerable variation about the mean was observed in individual subjects. The largest sialic acid and fucose values were seen in subjects with PK deficiency and sickle cell thalassemia disease. In these two diseases, the mean ratios of sialic acid to protein and fucose to protein were significantly reduced reflecting the elevated content of stromal protein. A direct correlation could be demonstrated between sialic acid and protein ($r = 0.9073$) and fucose and protein ($r = 0.6693$). These latter observations indicate that a quantitative relationship probably exists between individual components within the membrane mucopolysaccharide structures. There was no apparent correlation between analytical values and blood group phenotype.

The sialic acid content of normal human red cells is in close accord with the values reported by Eylar and co-workers\textsuperscript{12} and Yachnin and Gardner.\textsuperscript{4} Somewhat higher values have been reported by Manfredi\textsuperscript{15} and Mälkä and co-workers,\textsuperscript{16} and lower values have been reported by Yokoyama and Trams.\textsuperscript{17}
Table 1.—Hematologic Data and Erythrocyte Mucoid Composition in Different Anemias (Coombs' Negative)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis*</th>
<th>Phenotype</th>
<th>Reticulocytes %</th>
<th>Protein mg./ml. RBC</th>
<th>Sialic Acid µg./ml. RBC</th>
<th>Fucose µg./ml. RBC</th>
<th>Sialic Acid Protein µg./ml. RBC</th>
<th>Fucose Protein X 10³</th>
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<tbody>
<tr>
<td>Fl</td>
<td>Blood loss anemia</td>
<td>B+</td>
<td>15.7</td>
<td>11.2</td>
<td>131.2</td>
<td>19.1</td>
<td>6.9</td>
<td>11.7</td>
</tr>
<tr>
<td>Sp</td>
<td>PNH</td>
<td>O−</td>
<td>7.8</td>
<td>9.9</td>
<td>124.9</td>
<td>13.5</td>
<td>9.2</td>
<td>12.6</td>
</tr>
<tr>
<td>Sp</td>
<td>PNH</td>
<td>O+</td>
<td>9.0</td>
<td>9.6</td>
<td>121.5</td>
<td>14.5</td>
<td>8.1</td>
<td>12.6</td>
</tr>
<tr>
<td>Hu</td>
<td>PK deficiency</td>
<td>B+</td>
<td>35.0</td>
<td>14.5</td>
<td>175.5</td>
<td>20.5</td>
<td>8.5</td>
<td>12.1</td>
</tr>
<tr>
<td>Da</td>
<td>PA, partial remission</td>
<td></td>
<td>16.7</td>
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<td>117.5</td>
<td>14.2</td>
<td>9.5</td>
<td>12.3</td>
</tr>
<tr>
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<td></td>
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<td>16.5</td>
<td>8.1</td>
<td>13.2</td>
</tr>
<tr>
<td>Th</td>
<td>Sickle cell anemia</td>
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<td>7.4</td>
<td>14.1</td>
<td>140.0</td>
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<td>6.2</td>
<td>10.0</td>
</tr>
<tr>
<td>Br</td>
<td>PNH</td>
<td></td>
<td>4.2</td>
<td>8.6</td>
<td>114.1</td>
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<td>9.2</td>
<td>13.3</td>
</tr>
<tr>
<td>Po</td>
<td>ITP, blood loss</td>
<td>O−</td>
<td>4.6</td>
<td>10.7</td>
<td>118.5</td>
<td>24.7</td>
<td>10.7</td>
<td>11.1</td>
</tr>
<tr>
<td>Le</td>
<td>Blood loss anemia</td>
<td>O+</td>
<td>13.4</td>
<td>10.8</td>
<td>112.5</td>
<td>19.6</td>
<td>5.7</td>
<td>10.4</td>
</tr>
<tr>
<td>Le</td>
<td>Blood loss anemia</td>
<td>O+</td>
<td>7.5</td>
<td>9.1</td>
<td>118.1</td>
<td>17.9</td>
<td>6.6</td>
<td>13.0</td>
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<tr>
<td>O'D</td>
<td>Hereditary spherocytosis</td>
<td></td>
<td>6.8</td>
<td>9.0</td>
<td>124.2</td>
<td>13.4</td>
<td>9.3</td>
<td>13.8</td>
</tr>
</tbody>
</table>

| Mean† | S.D.  | 11.0 | ±9.1 | 11.2 | ±3.5 | 133.9 | ±23.0 | 17.8 | ±3.9 | 7.8 | ±1.4 | 12.1 | ±1.1 | 1.6 | ±0.3 |
| Normal (13 subjects) | | | | | | | | | | | | | | | |
| Mean | S.D.  | 8.0  | ±0.6 | 133.6 | ±9.9 | 16.2 | ±2.3 | 8.4 | ±1.1 | 16.7 | ±1.3 | 2.0 | ±0.3 |

*Abbreviations: PNH = paroxysmal nocturnal hemoglobinuria; PK = pyruvic-kinase; PA = pernicious anemia; ITP = idiopathic thrombocytopenia purpura; S.D. = standard deviation.
†The quantity of carbohydrate or protein can be expressed on a per red basis by multiplying the values per ml. packed RBC's by 1.03 x 10⁻¹⁰.
These data shown in Table 1 in subjects with various anemias are in general agreement with analytical values reported by Yachnin and Gardner in subjects with paroxysmal nocturnal hemoglobinuria, Brunetti and his associates in PK deficiency, and Brunetti and Puxeddu in thalassemia.

Mucoid Composition in Subjects with AHA (Coombs' Positive). The analytical results of 10 determinations in 7 subjects with AHA are shown in Table 2. The protein content ranged from 10.1 to 17.5 mg./ml. RBC, the mean value being 12.8 mg./ml. RBC. As noted in the control anemia group, the protein content paralleled the reticulocyte count. The sialic acid and fucose content were not significantly different from normal. The mean protein, sialic acid and fucose values and standard deviation about the means are compared in Table 2 with the control anemia subjects; it is of interest that no statistically significant differences were noted between these groups of patients.

As seen in Table 2 and also in Figures 1 and 2, these data did show a statistically significant decrease in the ratio of sialic acid to protein and fucose to protein in subjects with AHA as compared with the control anemia group. The largest decrease was observed in the fucose to protein ratio. Fucose values were well correlated with stromal protein (r = 0.8070), but a poor correlation was observed between sialic acid and protein (r = 0.1275).

An attempt was made to correlate serology, blood group phenotype, and mucoid composition in subjects with AHA. Comparison of subjects with warm and cold type of hemolytic disease revealed no differences in analytical values. These data suggested a greater incidence of blood group O, Rh-positive. The greater incidence of group O phenotype in subjects with AHA has been reported by Hunt and Lucia, although not confirmed by other studies.

Sialic Acid and Fucose Content of Isolated Leukocytes. A study was made of the possible contribution of WBC contamination to red cell mucoid composition. Jandl and Tomlinson have demonstrated leukocyte adherence to antibody-coated cells, and Bazin and Delaunay have shown a relatively high content of sialic acid in neutrophils. An approximation of leukocyte contamination was obtained from the ratio O.D. /O.D. of the thiorbarbituric acid chromophore (see Methods). Stroma obtained from subjects with AHA, and particularly subjects with chronic leukemia, evidenced appreciable concentrations of 2-deoxyribose derived from leukocytes. Chemical analysis of isolated leukocytes established the presence of relatively large content of mucopolysaccharide substances (Table 3). Leukocyte mucopolysaccharides were particularly rich in fucose (in relation to sialic acid) as compared with red cells. The ratio of sialic acid to fucose was approximately 2.5/1 for leukocytes and 8/1 for red cells (Table 1 and 2). Since the red-cell ratio of sialic acid to fucose was constant between normal subjects, AHA, and the control anemia groups, it was concluded that leukocyte contribution to red-cell mucoid composition was insignificant.

DISCUSSION

The changes observed in this study related to reticulocytosis deserve comment. An increase in stromal protein was seen in all subjects with reticulocyto-
Table 2.—Hematologic Data and Erythrocyte Mucoid Composition in Subjects with AHA (Coombs' Positive)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis*</th>
<th>Phenotype</th>
<th>Reticulocytes</th>
<th>Direct Coombs</th>
<th>Indirect Coombs</th>
<th>Cold agglutinin titer</th>
<th>Autoagglutination</th>
<th>Protein mg./ml. RBC</th>
<th>Sialic Acid μg./ml. RBC</th>
<th>Fucose μg./ml. RBC</th>
<th>Sialic Acid × 10^6</th>
<th>Fucose × 10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>IHA</td>
<td>O+</td>
<td>17.6</td>
<td>4+</td>
<td>0</td>
<td>n.t.</td>
<td>4+</td>
<td>12.4</td>
<td>132.5</td>
<td>15.6</td>
<td>8.5</td>
<td>10.7</td>
</tr>
<tr>
<td>Ma</td>
<td>RCS</td>
<td>O+</td>
<td>5.2</td>
<td>4+</td>
<td>0</td>
<td>1/4000</td>
<td>4+</td>
<td>12.4</td>
<td>142.5</td>
<td>17.8</td>
<td>8.0</td>
<td>11.5</td>
</tr>
<tr>
<td>Ma†</td>
<td>RCS</td>
<td>O+</td>
<td>14.5</td>
<td>4+</td>
<td>0</td>
<td>1/4000</td>
<td>4+</td>
<td>14.1</td>
<td>144.0</td>
<td>19.9</td>
<td>7.3</td>
<td>10.2</td>
</tr>
<tr>
<td>Ma‡</td>
<td>RCS</td>
<td>O+</td>
<td>2.6</td>
<td>4+</td>
<td>1+</td>
<td>1/4096</td>
<td>n.t.</td>
<td>10.8</td>
<td>114.1</td>
<td>11.2</td>
<td>10.2</td>
<td>10.6</td>
</tr>
<tr>
<td>Ko</td>
<td>CLL</td>
<td>O+</td>
<td>4.1</td>
<td>4+</td>
<td>1+</td>
<td>1/8</td>
<td>n.t.</td>
<td>10.2</td>
<td>121.0</td>
<td>12.1</td>
<td>10.0</td>
<td>11.9</td>
</tr>
<tr>
<td>Li‡</td>
<td>LE</td>
<td>O+</td>
<td>27.0</td>
<td>4+</td>
<td>4+</td>
<td>1/128</td>
<td>4+</td>
<td>17.4</td>
<td>142.3</td>
<td>19.4</td>
<td>7.3</td>
<td>8.2</td>
</tr>
<tr>
<td>Li</td>
<td>LE</td>
<td>O+</td>
<td>21.3</td>
<td>4+</td>
<td>4+</td>
<td>n.t.</td>
<td>4+</td>
<td>12.5</td>
<td>137.5</td>
<td>16.7</td>
<td>8.4</td>
<td>11.0</td>
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<tr>
<td>Fu</td>
<td>CLL</td>
<td>A+</td>
<td>29.0</td>
<td>3+</td>
<td>n.t.</td>
<td>0</td>
<td>n.t.</td>
<td>12.2</td>
<td>126.2</td>
<td>15.5</td>
<td>8.2</td>
<td>10.1</td>
</tr>
<tr>
<td>McM</td>
<td>CLL</td>
<td>O+</td>
<td>5.9</td>
<td>3+</td>
<td>0</td>
<td>n.t.</td>
<td>n.t.</td>
<td>10.1</td>
<td>121.5</td>
<td>12.4</td>
<td>10.1</td>
<td>11.2</td>
</tr>
<tr>
<td>Co</td>
<td>CD</td>
<td>A+</td>
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<td>4+</td>
<td>n.t.</td>
<td>1/160</td>
<td>4+</td>
<td>17.5</td>
<td>118.8</td>
<td>16.7</td>
<td>7.1</td>
<td>6.8</td>
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</table>

Mean***  
16.5  
±10.7

S.D.  
±2.6  
±7.8

Normal (13 subjects)

Mean

8.0

±0.6

±9.9

±2.3

±1.1

±1.3

±0.3

*Abbreviations: IHA = idiopathic hemolytic anemia; RCS = reticulum cell sarcoma; CLL = chronic lymphatic leukemia; LE = lupus erythematosus; CD = collagen disease; n.t. = not tested; S.D. = standard deviation.
†Modified procedure—sedimentation of red cells with PVP.
‡Iron deficiency present with serum iron of 18 μg. per cent and total iron-binding capacity of 280 μg. per cent.
§Post-transfusion and steroid therapy.
¶Differences compared with control anemia not considered significant (0.05 < p) (student "t" test).
††Differences compared with control anemia considered significant (0.02 < p < 0.05).
‡‡Differences compared with control anemia considered highly significant (0.001 < p < 0.005).
***The quantity of carbohydrate or protein can be expressed on a per red cell basis by multiplying the values per ml. packed RBC's by 1.01 × 10^-10.
Fig. 1.—Comparison of erythrocyte stromal sialic acid to protein ratio in 11 subjects with Coombs' negative hemolytic or blood loss anemia and in 7 subjects with acquired Coombs' positive hemolytic anemia. The mean and one standard deviation are indicated.

sis, whether Coombs' negative or positive. This increase in stromal protein appears to be related in part to an increase in red cell surface area associated with an increase in cell size of young red cells. Likewise, red cells with reduced mean corpuscular volume and mean corpuscular hemoglobin contain the largest quantity of stroma for each volume of packed red cells. These relationships were evident in subjects with markedly elevated reticulocyte counts (PK deficiency) and marked leptocytosis (sickle cell thalassemia); these subjects showed the highest values (Table 1). Similar changes in protein values were observed in a simple blood-loss anemia by Tishkoff and his associates. The changes in stromal protein may reflect, in part, the ribosomal protein and/or transferrin content of reticulocytes.

The data show again the previous findings of Yachnin and Gardner that reticulocytosis is associated with alteration in stromal carbohydrate composition. It is thus imperative to take into account the reticulocyte values when comparing changes in stromal carbohydrate composition which may occur in various types of hemolytic anemias. The data show that there is a change in mucoid composition in subjects with Coombs' positive hemolytic anemia; the
fucose to protein ratio was smaller in subjects with AHA than in normal or control anemia patients, with lesser changes observed in the sialic acid to protein ratio.

These data lend support to the possibility that Coombs’ positive red cells are biochemically different from Coombs’ negative cells. We consider it unlikely that the lower fucose to protein ratio observed in AHA subjects is attributable solely to the reticulocytosis (with resultant elevation of stromal protein values) for the following reasons: The mean reticulocyte counts and stromal protein values were identical in the two groups of subjects (Table 2). Furthermore, the differences observed in subjects with AHA were limited primarily to the fucose/protein ratio with little change in the sialic acid/protein ratio.

The differences cannot be explained by simple adsorption of proteins to the red cell membrane, whether derived from antibody, plasma proteins,24,25 or hemoglobin contamination.26,27 Adsorption of proteins of low carbohydrate content (i.e., hemoglobin) should result in a comparable decrease in sialic acid/protein ratios; that this was not observed would exclude appreciable adsorption of carbohydrate-poor protein. On the other hand, adsorption of
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Table 3.—Sialic Acid and Fucose Content of TCA-Soluble Extracts of Isolated Leukocytes

<table>
<thead>
<tr>
<th></th>
<th>Sialic Acid µg./ml. WBC</th>
<th>Fucose µg./ml. WBC</th>
<th>Sialic Acid Fucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes*</td>
<td>306</td>
<td>157</td>
<td>2.0</td>
</tr>
<tr>
<td>Granulocytes†</td>
<td>470</td>
<td>150</td>
<td>3.1</td>
</tr>
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</table>

*Isolated from a subject with chronic lymphocytic leukemia.
†Isolated from a subject with chronic granulocytic leukemia.

carbohydrate-rich proteins could conceivably alter the red-cell mucoid composition. The carbohydrate composition of the various globulins have been well characterized. The calculated fucose/protein ratio of γ G and γ M immunoglobulins are 2.2 and 2.9 × 10⁻³, respectively. Therefore, simple adsorption of globulins to the cell surface should result in an increase in the fucose/protein ratio. Furthermore, quantitative studies of red cell interaction with incomplete antibody have established the quantity of adsorbed globulins to be of the magnitude of micrograms per ml. of packed red cells, a quantity of protein which we consider unlikely to influence the red cell membrane mucoid composition.

The nature of the red cell lesion in AHA remains unexplained. Red cell autosurvival studies in subjects with AHA have failed to demonstrate a correlation between the quantity of cell coating globulins and red cell lifespan. Coombs’ positive cells may survive normally when transfused into normal recipients. Normal red cells sensitized in vitro with incomplete antibody may have a normal survival, although the sensitized cells evidence a strongly positive Coombs’ test. Not infrequently, splenectomy and corticosteroids induce a clinical remission, although the Coombs’ test remains positive. These observations strongly suggest that undetermined factors in addition to red cell coating globulins influence the regulation of red cell destruction.

The finding that the fucose/protein ratio (and to a lesser extent the sialic acid/protein ratio) is reduced—a finding which cannot be explained by reticulocytosis or nonspecific adsorption of globulins—suggests an alteration in the carbohydrate moiety of red cell membranes. This may be relevant to the shorter lifespan of the red cells. The changes observed in red cell mucosubstances could result from the selective action of enzymes such as proteases or mucopolysaccharases. It is also possible that certain proteases contained in the cell membrane may become activated. This concept would not be inconsistent with an underlying immunologic mechanism, since one mechanism of enzyme activation may result from interaction of antigen and antibody on the red cell surface structure.

Summary

Erythrocyte membrane mucoid composition has been quantitated in 13 normal subjects, 11 subjects with Coombs’ negative hemolytic or blood-loss anemia with reticulocytosis and 7 subjects with acquired autoimmune hemolytic anemia. The stromal protein value was increased in all subjects with anemia.
and reticulocytosis. The increase in protein paralleled the reticulocyte count. An abnormality of the erythrocyte mucoid composition was observed in acquired autoimmune hemolytic anemia as evidenced by a significant decrease in the fucose to protein ratio. A lesser change was observed in the sialic acid to protein ratio. It is speculated that these changes in membrane mucoid composition may be related to the selective modification of certain red cell, fucose-containing receptors by endogenous enzymes, possibly activated by antigen-antibody interaction.

SUMMARIO IN INTERLINGUA

Le composition mucoide del membrana erythrocytic esseva quantificate in 13 subjectos normal, in 11 subjectos con anemia hemolytic o a perdita de sanguine Coombs-negative e reticulocytosis, e in 7 subjectos con acquiretie anemia hemolytic autoimmun. In omne le subjectos con anemia e reticulocytosis, le valores pro proteina stromal esseva augmentate. Le augmentos in proteina esseva in parallela con le numerationes reticulocytic. Un anormalitate del composition mucoide del erythrocytos esseva observate in acquiretie anemia hemolytic autoimmun, evidentiate per un declino significative in le proportion de fucosa a proteina. Un minus marcate alteration esseva observate in le proportion de acido sialic a proteina. Es speculate que iste alterationes in le composition mucoide del membrana es possibilemente relationate con le modification selective de certe receptores erythrocytic a contento de fucosa sub le influentia de enzymas endogene le quales es forsan activate per le interaction de antigeno e anticorpore.

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ERYTHROCYTE MUCOIDS IN ACQUIRED HEMOLYTIC ANEMIA


Erythrocyte Mucoids in Acquired Autoimmune Hemolytic Anemia

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