Intracellular Protein Resembling Russell Bodies in Malignant Lymphomas Associated with Acquired Hemolytic Anemia

By Henry Rappaport and Frank B. Johnson

The relatively frequent association of acquired hemolytic anemia with malignant lymphomas of various types has been emphasized in recent reports. In many of these cases immune autoantibodies were demonstrated. Our own observations indicate that this association is far more common than can be explained on the basis of mere coincidence. While the site of antibody production in acquired hemolytic anemia associated with malignant lymphomas has not been ascertained, Dameshek and his associates have suggested that “the occurrence of hemolytic anemia in association with chronic lymphocytic leukemia, lymphosarcoma, and reticulum cell sarcoma may indicate that the proliferating abnormal tissue itself produces an abnormal globulin (antibody) which has the capacity to affix itself to the red blood cells and thus to cause their early destruction.” It is in connection with this hypothesis that our observations may be of interest.

Material and Methods

In the course of a study of the histopathologic features of acquired hemolytic anemia in 14 cases at the Armed Forces Institute of Pathology, an association with malignant lymphoma was noted in 14. Table I lists the types of lymphomas represented and the number of cases in which each type was observed. Eight patients of this group had a positive Coombs test. In three of these many of the component cells of the malignant lymphoma contained intracytoplasmic acidophilic inclusions resembling Russell bodies, and it is with the study of these cases that this report is primarily concerned. The acidophilic bodies were investigated for their tinctorial and histochemical properties, using most of the methods employed by Pearson in his study of the nature of Russell bodies and Kurloff bodies. Only formalin-fixed material was available. Paraffin sections were stained by the following methods: hematoxylin-eosin, Masson’s trichrome (Mallory modification), Weigert’s fibrin, phosphotungstic acid hematoxylin, crystal violet for amyloid, and aqueous solution of toluidine blue for metachromasia. Paraffin sections were also stained by the periodic acid-Schiff (PAS) method, with omission of the reducing rinse. The procedure was carried out on untreated sections as well as those treated with the following agents: diastase, hyaluronidase, methanol and chloroform in equal parts, and acetic anhydride-pyridine (2:3). Also employed were the method of Rinehart and Abul-Haj, intended for the demonstration of acid-mucopolysaccharides, and the Millon reaction (Benaley and Gersh) to confirm the protein nature of the intracytoplasmic material. Paraffin and frozen sections were stained for lipids with oil red O and sudan black B.

Control studies, employing the same staining and histochemical methods, were carried out on two groups of material. One was made up of sections from localized inflammatory lesions not associated with hemolytic anemia and containing many plasma cells with Russell bodies. They included nasal mucosa from patients with rhinoscleroma, and stomach mucosa diffusely infiltrated with plasma cells. The other group consisted of sections from

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Submitted May 20, 1954; accepted for publication June 22, 1954.

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Table 1.—Types of Malignant Lymphoma Associated with Acquired Hemolytic Anemia

<table>
<thead>
<tr>
<th>Classification</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>6</td>
</tr>
<tr>
<td>Hodgkin’s disease</td>
<td>3</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Reticulum cell sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Malignant lymphoma, follicular type</td>
<td>1</td>
</tr>
</tbody>
</table>

100 unselected cases of malignant lymphoma of various types, examined for the purpose of determining the incidence of Russell body-like material in a random sample of malignant lymphomas. The complete clinical records of 65 of these cases subsequently became available and were reviewed with particular reference to clinical and laboratory data suggestive or indicative of acquired hemolytic anemia.

**Clinical and Laboratory Data**

**Case 1.** (AFIP Acc. 497314). A 47 year old white man had a 6 year history of chronic lymphocytic leukemia before the appearance of severe anemia that failed to respond to transfusions of whole blood and washed red blood cells. There was no history of previous x-ray treatment, chemotherapy, or blood transfusions. The spleen was greatly enlarged, firm and not tender; the liver was moderately enlarged; significant enlargement of superficial lymph nodes was not observed. The red blood cell count on admission was 1,850,000, hemoglobin 5.9 Gm., hematocrit 19. Leukocyte count ranged between 18,500 and 57,000, with lymphocytes accounting for as much as 97 per cent. The highest reticulocyte count recorded was 4.4 per cent. Fecal urobilinogen was 446 mg. in 24 hours. Direct and indirect Coombs tests were positive on three occasions. Agglutinin titers, using cells of the same group and Rh type as those of the patient (OCDE), were determined before and during cortisone therapy (initial doses of 200 mg. followed by 100 mg. daily for 18 days) and are recorded in text Figure 1. The highest titers of warm agglutinins (37 C.) were 1:25,000 in albumin and 1:6400 in saline; the highest titer of cold agglutinins (5 C.) was 1:32,768 in albumin and saline. Following cortisone therapy the Coombs test became negative and agglutinins were no longer demonstrable. With blood transfusions the red cell count rose gradually to 4,400,000 and the hemoglobin to 14.6 Gm. Subsequently the anemia slowly but steadily became more severe and failed to respond to transfusion therapy. The patient died following a convulsive seizure, six months after onset of the hemolytic anemia. Postmortem examination confirmed the diagnosis of chronic lymphocytic leukemia.

**Case 2.** (AFIP Acc. 335892). A 32 year old white man complained of easy fatigability, weakness and loss of weight. He had become extremely weak following a recent episode of “flu” with high fever. On physical examination the spleen was found to be enlarged to the level of the umbilicus. Axillary, right epitrochlear and inguinal lymph nodes were slightly to moderately enlarged. The liver was unquestionably palpable one to two fingerbreadths below the right costal margin. The red blood count was 2,490,000, hemoglobin 12 Gm., hematocrit 39. White blood cell count was 5200, with 46 per cent segmented neutrophils, 2 per cent stab cells, 1 per cent eosinophils, 1 per cent monocytes, and 50 per cent lymphocytes. The platelets numbered 56,300. Total protein was 7.8 Gm., with albumin 3.5 Gm. and globulin 4.3 Gm. per cent. Cryoglobulins and a strongly positive formol gel test were also present. Spontaneous agglutination of the red cells was noted on several occasions when red cell counts were attempted. Fecal urobilinogen ranged from 171 to 398 mg. in 24 hours. Warm autoclaved agglutinin titers, using albumin as a diluent, showed variations between 1:256 and 1:8000. The direct Coombs test was positive on three occasions. Biopsy of an axillary lymph node failed to reveal conclusive evidence of malignant lymphoma. A subsequent biopsy of an epitrochlear lymph node was highly suggestive of malignant lymphoma, lymphocytic type. Laparotomy disclosed a large spleen reaching down to the left iliac crest and many large, soft, rather pale and discrete lymph nodes about the tail of the pancreas. Sections from these lymph nodes and the spleen revealed malignant lymphoma, lymphocytic type.
The neoplastic lymphocytes were well differentiated. There was moderate lymphocytosis in the peripheral blood. No significant change in the patient's condition had occurred when he was examined four months after splenectomy. At that time two large irregular masses were palpable in the lower abdomen. The total protein was 9.5, albumin 3.7 and globulin 5.8 Gm. per cent. The patient was still living one year after splenectomy had been performed.

Case 3. (AFIP Acc. 327642). A 53 year old colored man had become acutely ill in June 1948, with chills, fever, pallor, jaundice, dyspnea and tachycardia. On admission, the veins of the neck were distended, the spleen was much larger than normal, the liver was somewhat enlarged, and the lymph nodes slightly enlarged. The red blood cell count was 1,100,000, hemoglobin 3 Gm.; the white cell count was 73,000, with a differential count of 22 per cent segmented neutrophils, 31 per cent lymphocytes, 1 per cent monocytes, 44 per cent blast cells and 1 per cent promyelocytes. There were 9 nucleated red cells per 100 white blood cells. The icterus index was 27. The bone marrow showed hyperplasia, predominantly erythroid. The diagnosis of acute leukemia was made and the patient was treated with blood transfusions and urethane (4 Gm. daily for 14 days), following which the white cell count ranged from 4,100 to 8,900, with 40 to 54 per cent lymphocytes. The red cell count gradually rose to 4,000,000 in September 1949. The patient was relatively asymptomatic until January, 1950. At that time there appeared to be some doubt as to the diagnosis of acute leukemia. In July 1950, severe anemia recurred and was recognized to be hemolytic in type. The total serum bilirubin ranged between 0.75 and 2.05 mg. per cent. The direct Coombs test was consistently positive; reticulocyte count ranged between 2 and 7 per cent, and the anemia progressed in spite of blood transfusions. Axillary lymph node biopsy revealed a malignant lymphoma of the follicular type. Deep x-ray therapy to the spleen was ineffectual, while cortisone therapy (100 mg. twice a day for 20 days and 50 mg. twice a day for another 8 days) produced temporary improvement in the anemia, with the reticulocyte count rising to 21 per cent. The Coombs test was repeatedly positive during and after cortisone therapy, except for one occasion on the fifteenth day of therapy when it was negative. Splenectomy
was performed in December, 1950. After the operation, the red blood cell count slowly rose to 3,600,000, with hemoglobin of 11 Gm. The immediate result of splenectomy was considered good. Sections from both spleen and splenic lymph node showed malignant lymphoma of the follicular type. The Coombs test remained positive after splenectomy. The patient returned to the hospital April 10, 1951 with recurrence of anemia. The total white cell count was 39,200 with 12 per cent blast cells, 6 per cent myelocytes, 5 per cent promyelocytes, 40 per cent polymorphonuclear neutrophilic leukocytes. There were 81 nucleated red cells per 100 white cells. Frequent blood transfusions produced only transient increase in erythrocytes, and the patient became progressively worse and died April 25, 1951 following a short period of severe dyspnea, fever and physical manifestations suggesting pulmonary infarction. No autopsy was performed.

**Pathologic Observations**

A detailed account of the histopathologic findings in the surgical and post-mortem material of patients with acquired hemolytic anemia will be given in a separate communication. The pathologic studies reported here are concerned with the morphologic features, localization, and tinctorial and histochemical properties of the Russell body-like intracytoplasmic acidophilic material that attracted our attention to this group of cases. For the sake of brevity it will henceforth be referred to as RBLM.

In hematoxylin-eosin stained sections this material appeared either as a substance that imparted a strongly acidophilic staining property to the cytoplasm, or in the form of acidophilic globules with more or less well defined contours (plate I-3). Usually a single globule occupied almost the entire cell body, pushing the nucleus to the periphery (plate I-1, 3). The globules were usually not as round and as sharply defined as typical Russell bodies of plasma cells (plate I-2). This is the reason why we were reluctant to call them Russell bodies, in spite of the close similarities that otherwise exist. An occasional globule had a somewhat more lightly stained peripheral zone. We have not been able to observe a peripheral basophilic rim either in Russell bodies of plasma cells or in the Russell body-like globules in the lymphomatous tissue.

The RBLM was present in cells of two types: (1) neoplastic lymphocytes, and (2) histiocytes (macrophages) which did not appear to be part of the neoplastic process. In case 1 (chronic lymphocytic leukemia) the histologic picture was unusually striking. Many cells with either a single large globule or with strongly acidophilic cytoplasm were found not only in the lymph nodes and bone marrow, but also in practically all visceral leukemic infiltrations. The nuclei of the cells containing this material were indistinguishable from those of the other leukemic lymphocytes infiltrating the tissues. These cells lacked the nuclear characteristics of plasma cells, but some had a superficial resemblance to plasma cells because of the eccentric position of the nuclei. In addition, histiocytes containing Russell body-like globules were seen in the bone marrow. Large numbers of such histiocytic cells were found in the lymph node sinuses of the first biopsy specimen from case 2, in which the co-existing malignant lymphoma was at first not clearly recognized (plate I-8). In this as well as in subsequent biopsies, RBLM was also seen in the neoplastic lymphocytes within the medullary cords and in the spleen (plate I-7). In case 3, Russell body-like globules were more prominent in intra-sinusoidal histiocytes than in the neoplastic lymphocytes but were less abundant than in the two preceding cases.
The tinctorial properties of RBLM were practically identical with those of Russell bodies of plasma cells. The acidophilia in hematoxylin–eosin sections has already been pointed out. With Weigert's method for fibrin the staining varied between blue and purple, and with the phosphotungstic acid–hematoxylin stain, a dark purple color, sometimes with a brownish tinge, was obtained (plate 1-4). Masson stains (Mallory modification) gave an orange color (plate 1-5). Crystal violet stains for amyloid showed no metachromasia.

The Russell body-like material was particularly conspicuous in sections treated by the periodic acid–Schiff method (Plate 1-1, 7, 9). Often the cytoplasm of the neoplastic lymphocytes was abundant and showed a positive PAS reaction with considerable variation in staining intensity, ranging from pale pink to deep red (Plate 1-7). In the same areas well defined, intracellular, PAS-positive globules were seen. No comparable stages of transition were seen in macrophages, in which the RBLM always was present in the form of globules (Plate 1-8, 9).

The histochemical reactions are listed in table 2. The Russell body-like material is not affected by either diastase or hyaluronidase, lacks metachromatic properties, and is not altered by a mixture of methanol and chloroform. It gives a positive Millon reaction (Plate 1-6) and is negative for fat with oil red O and sudan black B. The results of these reactions indicate that it consists of a polysaccharide-containing protein and that it is histochemically very similar to, if not identical with, the Russell bodies of plasma cells.5

It is worthy of note that the plasma within the blood vessels of the spleen in Case 1 and of the spleen and lymph nodes of Case 2 showed a more pronounced acidophilia and also a more intense PAS reaction than are commonly observed in surgical and postmortem material. In the lymph nodes of Case 2, the PAS re-
INTRACELLULAR PROTEIN IN MALIGNANT LYMPHOMAS

TABLE 2.—Histochemical Reactions of Russell Body-like Material in Malignant Lymphomas

<table>
<thead>
<tr>
<th>Periodic acid-Schiff reaction</th>
<th>Positive</th>
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<tbody>
<tr>
<td>PAS after diastase</td>
<td>No decrease in staining intensity</td>
</tr>
<tr>
<td>PAS after hyaluronidase</td>
<td>No decrease in staining intensity</td>
</tr>
<tr>
<td>PAS after methanol and chloroform</td>
<td>No decrease in staining intensity</td>
</tr>
<tr>
<td>PAS after acetic anhydride-pyridine</td>
<td>Negative</td>
</tr>
<tr>
<td>Acid-mucopolysaccharides</td>
<td>Negative</td>
</tr>
<tr>
<td>Millon’s reaction</td>
<td>Positive</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>No metachromasia</td>
</tr>
<tr>
<td>Oil red O</td>
<td>Negative</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>Negative</td>
</tr>
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TABLE 3.—Pertinent Data in 7 Cases of Malignant Lymphoma (Control Group) in which Russell Body-like Material was Present in the Neoplastic Cells

<table>
<thead>
<tr>
<th>Acc. No.</th>
<th>Type of Lymphoma</th>
<th>Red Cell Count (millions per cu. mm.)</th>
<th>Protein</th>
<th>Serum Bilirubin</th>
<th>Reticuloocytes (per cent)</th>
<th>Clinical Icterus</th>
<th>Marked Anemia In Spite of Transfusions</th>
<th>Hemosiderin In Lymph Node Sections</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>232475</td>
<td>Chronic lymphoblastic leukemia</td>
<td>3.7 2.7 4.2</td>
<td>Alb (Gm/100cc.)</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>Erythroid hyperplasia of bone marrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>235166</td>
<td>Chronic lymphoblastic leukemia</td>
<td>1.1 2.0 8.9</td>
<td>Glob (Gm/100cc.)</td>
<td>0.13</td>
<td>0.50</td>
<td>0</td>
<td>Cryoglobulinemia, Urinary urobilinogen 1:200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>309806</td>
<td>Chronic lymphoblastic leukemia</td>
<td>4.1 4.5 1.9</td>
<td>Total (mg/100 cc.)</td>
<td>0.45</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>331366</td>
<td>Lymphosarcoma</td>
<td>2.5 3.0 3.4</td>
<td>Direct (mg/100 cc.)</td>
<td>0.40</td>
<td>0</td>
<td>0</td>
<td>Spleenectomy done because patient’s anemia was attributed to “blood destruction”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>501153</td>
<td>Chronic lymphoblastic leukemia</td>
<td>3.8 4.7 2.2</td>
<td>Alb (Gm/100cc.)</td>
<td>1.00</td>
<td>0.9</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>508007</td>
<td>Reticulum cell sarcoma</td>
<td>3.9</td>
<td>Alb (Gm/100cc.)</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
<td>Pronounced erythrophagocytosis in lymph node biopsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>605470</td>
<td>Malignant lymphoma, follicular type</td>
<td>2.4 3.7 3.6</td>
<td>Alb (Gm/100cc.)</td>
<td>0.0</td>
<td>2.2</td>
<td>0</td>
<td>Spleen consistent with hemolytic anemia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lymph node biopsy preceded by blood transfusions.
† Lymph node biopsy not preceded by blood transfusions.
‡ This histologic observation in conjunction with hemosiderosis is regarded as strongly suggestive of hemolytic anemia since the lymph node biopsy was not preceded by blood transfusions or other therapy.

action of the blood plasma was almost as strong as that of the globules within macrophages. This might well be related to the high globulin level in the patient’s plasma.

In the control group of 65 unselected cases of malignant lymphoma of various types in which sufficient clinical and laboratory data were available, significant amounts of intracellular Russell body-like material were noted in 7. Clinical and laboratory data pertaining to the seven cases are assembled in table 3. They
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indicate that an elevated serum globulin, as determined by routine laboratory methods, was present in four patients, one of whom had definite, and two suggestive, evidence of hemolytic anemia. The lymph node of a fifth patient exhibited pronounced erythrophagocytosis, regarded as strongly suggestive of hemolytic anemia since the lymph node biopsy was not preceded by blood transfusions or any other form of therapy. In contrast, of the 58 cases in which RBLM was not demonstrated, there were only five in which evidence of an elevated serum globulin was observed. The diagnosis of hemolytic anemia could be neither suggested nor established in any of these 58 cases on the basis of available information. In most of them, however, clinical and hematologic data, while generally adequate, were not sufficient to exclude hemolytic anemia with certainty.

DISCUSSION

The significant observations in our 3 cases of malignant lymphoma associated with acquired hemolytic anemia may be summarized as follows: (1) large amounts of intracellular protein closely resembling Russell bodies tinctorially and histochemically were observed in the lymphomatous tissue. (2) This material was found in neoplastic lymphocytes and in non-neoplastic histiocytes (macrophages). (3) In neoplastic lymphocytes intermediate stages were seen between cells with cytoplasm giving a faintly positive PAS reaction and others containing fully developed Russell body-like globules which were strongly PAS positive. (4) In the macrophages the material was present in the form of globules without appreciable variations in the intensity of the PAS reaction.

It would be of considerable interest to determine whether our observations have any bearing on the possible site of formation of abnormal proteins in malignant lymphomas in general, or of antibodies in certain malignant lymphomas associated with acquired hemolytic anemia in particular, for the following reasons: (1) The reticuloendothelial system and more specifically the lymphocytes have been implicated as probable sites of antibody formation. (2) Some observers hold that the plasma cell and not the lymphocyte is the cellular source of antibody. (3) Pearse demonstrated that the Russell bodies of plasma cells are mucoproteins and suggested that these mucoproteins are polysaccharide-containing globulins related in some way to antibodies. While the divergent views as to the specific cell types concerned with antibody production have not been reconciled, it may be significant that we were able to demonstrate polysaccharide-containing protein not only in plasma cells but also in lymphocytes, albeit neoplastic ones. If one accepts the hypothesis that normal lymphocytes may form antibodies, it seems reasonable to suggest that neoplastic lymphocytes, too, may be functioning cells and capable of forming abnormal protein which may or may not be antibody. This would be comparable to the evidence of function exhibited by other neoplastic cells derived from the reticuloendothelial system, e.g., phagocytosis and production of reticulin in some types of reticulum cell sarcomas and formation of Bence-Jones protein by multiple myeloma cells.

The suggestion made by Dameshek and his associates that the malignant lymphoma tissue itself may be the site of production of abnormal proteins and possibly of antibody in patients with malignant lymphoma and acquired hemolytic anemia was based primarily upon clinical observations, including the effects
of ACTH which simultaneously produced decrease in the autoagglutinin titers and peripheral lymphocytes, regression of lymph node size, and clinical improvement. Craig, Waterhouse and Young also reported reduction in the number of circulating lymphocytes, in the size of lymph nodes, and in the rate of red cell destruction following administration of ACTH in a case of autoimmune hemolytic disease and cryoglobulinemia associated with chronic lymphocytic leukemia.

More direct evidence in support of formation of an abnormal protein by malignant lymphoma tissue was brought forth by Abrams, Cohen and Meyer, who extracted from the tissue of lymph nodes a cryoglobulin which had properties identical with those of a circulating cryoglobulin in the patient's blood serum. While they recognized that the presence of a protein in a tissue does not establish that tissue as the site of formation, they submitted evidence which strongly suggests that the cryoglobulin was actually derived from the lymphosarcoma cells.

It is of interest in this connection that an abnormal protein with the properties of cryoglobulin was present in case 2 of our original series and in one case of the control group (see table 3). In both cases many of the neoplastic cells contained intracellular PAS-positive protein (RBLM).

Electrophoretic analyses of the serum and plasma proteins in patients with malignant lymphoma have been carried out by several workers. Increase in the gamma globulin was noted in some cases of Hodgkin's disease, chronic lymphocytic leukemia and reticulum cell sarcoma. The findings were neither consistent nor specific and no data permitting a correlation between the gamma globulin levels and the presence or absence of hemolytic anemia are available.

The question arises whether the demonstration of RBLM in the neoplastic cells of malignant lymphomas actually means that this substance is formed rather than taken up by the cells in which it is contained. Pearse, in his study on the Russell bodies of plasma cells, described intermediate stages between cells with weakly PAS-positive cytoplasm and those with deeply staining, fully developed Russell bodies; comparison with sections stained by the Unna-Pappenheim method showed that the amount of pyronin-positive material (ribonucleic acid) decreases as the amount of PAS-positive substance (mucoprotein) rises. Pearse concluded that this inverse relationship between ribonucleic acid and mucoprotein supports synthesis and not absorption. Lack of suitably fixed material prevented us from performing adequate methyl green pyronin stains. We believe, however, that the presence of intermediate stages between weakly PAS-positive cytoplasm and the fully developed Russell body-like globules strongly favors the interpretation that the RBLM is formed by the neoplastic lymphocytes in which it is contained. This is in accord with the generally accepted view that lymphocytes do not phagocytize. In contrast, the Russell body-like globules in the macrophages showed no appreciable variations in the intensity of the PAS reaction. On the basis of these observations, we suggest that in our cases of malignant lymphoma associated with acquired hemolytic anemia, the PAS-positive material was formed by the neoplastic lymphocytes and taken up by macrophages.

Two possible interpretations of our observations are suggested: (1) That RBLM may represent an abnormal protein (globulin) produced by neoplastic lymphocytes in response to an unknown stimulus, released into the circulation, and eventually adsorbed to the surface of red cells. Some of the breakdown prod-
products of these altered red cells may act as antigens leading to the production of anti-red cell antibody. (2) That the intracellular PAS-positive substance may, in some instances, be a morphologic manifestation of the production of abnormal globulins representing the antibody itself. Further studies would seem indicated to ascertain if either of these hypotheses can be substantiated. It might be possible, for instance, to demonstrate antigenic material within malignant lymphoma cells by means of fluorescein antibody conjugates, using fresh biopsy material and antibody-containing serum from the same patient, an experiment which we were unable to carry out.

Thus far we have been unable to explain to our satisfaction why RBLM was found in only three of the eight patients who had malignant lymphoma and hemolytic anemia with demonstrable antibodies and not in any of those with idiopathic acquired hemolytic anemia. It appears likely that intracellular accumulation of RBLM, and particularly its retention in globular form, will take place only when release of this substance cannot keep pace with its production. This situation might exist whenever production is excessive. It was, therefore, thought that in two of our cases the presence of intracellular RBLM might be accounted for by the unusually high autoagglutinin titers (1:8096 and 1:25000 respectively). No agglutinin titers of comparable height were obtained in the remaining cases of symptomatic acquired hemolytic anemia or in any patient with the idiopathic form of the disease. Since the agglutinin titer may afford at least some measure of the quantity of antibody production, our failure to demonstrate RBLM in any case of idiopathic acquired hemolytic anemia in our series might be related to the comparatively low autoagglutinin titers in this particular group. It is also known that autoagglutinin titers in acquired hemolytic anemia are subject to considerable fluctuation. If similar fluctuation exists in the amount of abnormal protein production, it is conceivable that intracellular retention of RBLM may be transient and not demonstrable at all times in the course of a patient’s illness.

No claim is made that the presence of intracellular PAS-positive protein in malignant lymphoma necessarily indicates that the patient has a hemolytic disorder. It merely suggests production of abnormal proteins, as brought out by the study of a control group of unselected cases of malignant lymphoma. The reason for our failure to find conclusive evidence of hemolytic anemia in the records of some of these patients (see table 3) cannot be accurately assessed, since the more sensitive methods for the diagnosis of hemolytic anemia, such as red cell survival, fecal urobilinogen, reticulocyte counts and pertinent serologic studies, were not carried out in most instances. A second factor to be considered is that it is not known how frequently circulating abnormal proteins induce the sequence of events leading to the development of acquired hemolytic anemia. Careful study of large numbers of patients with malignant lymphomas, using laboratory methods sensitive enough to detect subclinical hemolytic anemia, as well as histochemical procedures to demonstrate RBLM, may offer more conclusive answers to some of the questions raised by our findings.

**Summary**

Large amounts of intracellular periodic acid-Schiff positive protein, closely resembling Russell bodies tinctorially and histochemically and, to varying degrees, morphologically, were found in the neoplastic cells of three patients with
malignant lymphoma associated with acquired hemolytic anemia. Direct Coombs tests were positive in all three of these patients, and in two of them, immune autoantibodies against red cells were observed in unusually high titers. One of the latter also had abnormally high serum globulin levels with cryoglobulinemia. A similar intracellular protein was present in the neoplastic tissue of seven of a control group of 65 unselected cases of malignant lymphoma. The serum globulin was elevated in four of the seven patients and one of these also had cryoglobulinemia. Definite evidence of hemolytic anemia was noted in one, and suggestive evidence in two of these four patients. A lymph node removed from a fifth patient, on whom no serum protein determinations had been made, showed hemosiderosis and marked erythrophagocytosis. This patient had not received blood transfusions or other treatment before biopsy was performed.

On the basis of our observations, it is suggested (1) that under certain conditions the neoplastic cells of malignant lymphomas are capable of synthesizing abnormal proteins; (2) that the presence of Russell body-like intracellular PAS-positive material in the neoplastic cells may be the morphologic manifestation of this protein synthesis; (3) that these abnormal proteins may play an important part in the immunological mechanisms responsible for the development of acquired hemolytic anemia in association with malignant lymphomas.

**SUMMARIO IN INTERLINGUA**

Esseva trovate, in le cellulas neoplastic de tres patientes con maligne lymphomas associate con acquirite anemia hemolytic, grande quantitates de proteina intracellular que reageva positivemente al periodic reaction acide Schiff e resimilava fortemente le corpores de Russell tanto tinctorial- e histochimicamente como etiam in varie grades morphologicamente. Le directe reaction de Coombs esseva positive in omne le tres patientes. In duo de illes autoanticorpores immunisante contra erythrocytos esseva observate in titros inusualmente alte. In un del patientes de iste ultime grupo anormalmente alte nivellos de globulina seral esseva observate insimul con cryoglobulinemia.

Un simile typo de proteina intracellular esseva presente in le texito neoplastic de 7 inter 65 non-seligite casos de maligne lymphomas que serviva como grupo de controlo. Le globulina seral esseva elevate in quatro de iste 7 patientes, e un de illes etiam habeva cryoglobulinemia. Evidentia univoc de anemia hemolytic esseva observate in un de illes e signos suspecte in duo alteres. Un nodo lymphatic removite ab un quinte paciente in cui caso nulle determination de proteina seral habeva esse operata monstrava hemosiderosis e marcate erythrophagocytosis. Iste paciente non habeva habite transfusiones de sanguine o alte formas de tractamento ante le execution del biopsia.

Super le base de nostre observationes nos conclude (1) que sub certe conditiones le cellulas neoplastic de maligne lymphomas es capace a synthetisar proteinas anormal; (2) que le presentia in le cellulas neoplastic de un substantia intracellular que resimila corpores de Russell e es positive al periodic reaction acide Schiff pote esser le manifestation morphologic de iste synthese de proteina; e (3) que tal formas anormal de proteina ha possibilemente un importante function in le mechanismos immunologic que es responsabile pro le disveloppamento de acquirite anemia hemolytic in association con maligne lymphomas.
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intracellular protein in malignant lymphomas


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