L.E. Cells in Lymphoma

By June Howqua and Ian R. Mackay

THIS REPORT DESCRIBES the finding of positive clot tests for lupus erythematosus (L.E.) cells in two women suffering from lymphoma—lymphosarcoma and Hodgkin's disease. These observations have particular relevance to Burnet's concept of autoantibody production by “forbidden” clones of lymphoid cells.

METHODS

The L.E. cell test was performed by the 2-hour clot method. Antinuclear factor was tested for by fluorescence microscopy of a blood film treated with the patient's undiluted serum and then fluorescein-labeled rabbit anti-human gamma globulin. Anticytoplasmic autoantibodies were tested for by complement fixation (C.F.) using fresh human liver as antigen; titers of 1/16 or higher were considered significantly positive. Antithyroglobulin antibody and rheumatoid factor were tested for by tanned red cell hemagglutination, the cells being coated with thyroglobulin and heat-denatured gamma globulin respectively; titers of 1/100 or higher were regarded as significantly positive.

CASE REPORTS

Case 1

K. D., a female aged 65, was admitted to hospital in November, 1960, with fever and right-sided chest pain of 10 days' duration. Her previous illnesses had included an attack of jaundice when aged 10 years, recurrent gall stone dyspepsia, and an episode of bleeding from a duodenal ulcer in 1955. Her admission illness was diagnosed as bilateral basal bronchopneumonia. In addition she had enlarged nodes in the axillae and groins, enlargement of the liver and spleen and slight proteinuria. Resolution of the pneumonia was slow and incomplete and her fever persisted despite treatment with tetracycline. A macular rash lasting 6 days appeared on the face, forearms and legs 10 days after admission.

Laboratory findings. These are presented in detail in Table 1. The initial leukocyte count of 7000 cells per cu.mm. included 15 per cent eosinophils. The serum gamma globulin level, initially 4.1 Gm. per 100 ml., was electrophoretically heterogeneous and ultracentrifuge analysis did not reveal an excess of macroglobulins. The L.E. cell preparation contained numerous typical L.E. cells, and all of the 24 tests performed throughout her illness were positive. The C.F. test with liver antigen was weakly positive, giving titers in the range of 1/4-1/16 throughout the illness; the serum was frequently anticomplementary. The titer of antithyroglobulin antibody ranged from 1/3400 to 1/20000, and the titer of rheumatoid factor was 1/2500 on a single examination. Fluctuations in titer in these serologic reactions had no apparent relation to phases of the illness or to treatment given.

Biopsies. Biopsy of an axillary lymph node showed diffuse replacement of the normal follicular pattern by sheets of small lymphocytes (fig. 1). Liver biopsy revealed dense focal collections of small lymphocytes located mainly in the portal tracts (fig. 2); treatment

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### Table 1.—Summarized Laboratory Data for Cases 1 and 2

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Gm. per 100 ml.)</td>
<td>11.6-7.6</td>
<td>12.5-6.8</td>
</tr>
<tr>
<td>Leukocyte count per cu. mm.</td>
<td>12,500-3,500</td>
<td>30,000-16,000</td>
</tr>
<tr>
<td>Differential leukocyte count</td>
<td>unremarkable</td>
<td>neutrophilia</td>
</tr>
<tr>
<td>Platelet count per cu. mm.</td>
<td>160,000-34,000</td>
<td>355,000</td>
</tr>
<tr>
<td>Bone marrow smear</td>
<td>40% small lymphocytes</td>
<td>active granulopoiesis</td>
</tr>
<tr>
<td>Serum albumin (Gm. per 100 ml.)</td>
<td>3.9-2.4</td>
<td>3.2-2.7</td>
</tr>
<tr>
<td>Serum globulin (Gm. per 100 ml.)</td>
<td>6.6-4.3</td>
<td>3.9-2.5</td>
</tr>
<tr>
<td>Serum gamma globulin (Gm. per 100 ml.)</td>
<td>4.1-2.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Serum bilirubin (mg. per 100 ml.)</td>
<td>2.4-0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Serum hexosamine (mg. per 100 ml.)</td>
<td>105-118</td>
<td>144</td>
</tr>
<tr>
<td>Blood urea (mg. per 100 ml.)</td>
<td>66-30</td>
<td>27</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm. per hr. Westergren)</td>
<td>100-54</td>
<td></td>
</tr>
<tr>
<td>L.E. cell preparation</td>
<td>+ve consistently</td>
<td>negative X6, doubtful positive X2, positive X2.</td>
</tr>
<tr>
<td>Anti-nuclear factor immunofluorescence technie</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Anticytoplasmic antibody—human liver antigen—complement fixation titer</td>
<td>16-4</td>
<td>-ve</td>
</tr>
<tr>
<td>Antithyroglobulin antibody—tanned cell hmagglutination titer</td>
<td>20,000-3,400</td>
<td>-ve</td>
</tr>
<tr>
<td>Rheumatoid factor—tanned cell hmagglutination titer</td>
<td>2,500</td>
<td>-ve</td>
</tr>
<tr>
<td>Direct Coombs reaction</td>
<td>- (terminally)</td>
<td>+</td>
</tr>
</tbody>
</table>

Treatment. Initial treatment with prednisolone, 40 mg. daily, was withdrawn after 7 days owing to severe abdominal pain; it caused a temporary fall in the temperature, erythrocyte sedimentation rate (from 130 mm. to 40 mm. per hour), and serum hexosamine level (from 165 to 136 mg. per 100 ml.), but she remained unwell. The histologic diagnosis of lymphosarcoma prompted the use of P³², 6 mc. being given in three divided doses over 4 weeks in February, 1961. This resulted in pronounced clinical improvement, weight gain, disappearance of fever, and shrinkage of the enlarged lymph nodes; within 4 weeks the gamma globulin fell from 4.1 to 2.4 Gm. per 100 ml., the L.E. cell preparation showed only scanty numbers of L.E. cells and, as stated, a second liver biopsy showed a decrease in the lymphoid cell infiltration.

Progress. In May, 1961, the gamma globulin level had risen to 3.8 Gm. per 100 ml. and the L.E. cell preparation again showed numerous L.E. cells, and two further courses of P³² of 1 mc. orally were given in August and December. These were followed by improved health and a diminution in the number of L.E. cells in the L.E. preparation. The patient was readmitted to hospital with suppurative mastitis in October, 1961, and with left upper abdominal pain suggestive of a splenic infarct in December, 1961.

Thereafter her health declined. She developed a refractory anemia with hemoglobin levels between 7 and 8 Gm. per 100 ml. despite transfusion, and leukopenia, and thrombocytopenia, with platelet levels falling to 34,000 per cu.mm. Bone marrow biopsy showed hyperactive erythropoiesis and a lymphocytosis of 40 per cent. A 6-week course of adrenocorticotropic hormone, 40 units on alternate days, failed to effect improvement. A radiochromium study in May, 1962 showed the half-life of the labeled cells to be 15 days, and surface counting over the spleen revealed excessive counts between days 6 and 23. It was concluded that she had a moderately severe hemolytic process associated with some degree of bone marrow depression: the latter was attributed to the combined effects on the marrow of lymphosarcomatous infiltration and treatment with P³². She died in July, 1962, following an episode of severe abdominal pain, shock, and peripheral circulatory failure.
Fig. 1.—Axillary lymph node biopsy (Case 1) showing replacement of architecture by sheets of lymphocytes and infiltration of the surrounding fat (lower center). HE x 40.

Fig. 2.—Liver biopsy at onset of illness (Case 1) showing a focal collection of small lymphocytes. HE x 220.

Necropsy findings. The significant macroscopic findings included skin purpura, numerous petechial hemorrhages on the serosal surfaces, particularly over the small intestine, an enlarged liver (1250 Gm.), the cut surface of which showed small foci of infiltrating tissue, an enlarged spleen (625 Gm.), and enlarged discrete thoracic and abdominal lymph nodes, up to 2.5 cm. in diameter.

Microscopically the liver was extensively infiltrated with masses of small lymphocytes (fig. 3). Discrete foci of small lymphocytes were present in the bone marrow (fig. 4), the kidney and the lung. The thyroid gland showed colloid goiter with pronounced lymphoid
infiltration. The thymus was very atrophic but otherwise unremarkable. The abdominal lymph nodes showed obliteration of the follicular structure by small lymphocytes. Hematoxylin bodies were not detected. The microscopic appearances were those of lymphocytic lymphosarcoma.

Case 2

J. A., a female aged 48, presented in October, 1958, with malaise and vague generalized pains of 2 week's duration. The physical findings included pallor, moderately enlarged axillary lymph nodes and swollen tender interphalangeal and knee joints. The initial diagnosis
Fig. 5.—Necropsy section of spleen (Case 2) showing appearances of Hodgkin's disease with numerous Sternberg-Reed giant cells. HE x 250.

of acute rheumatoid arthritis was not sustained after admission to hospital. Treatment, including penicillin, tetracycline, nitrofurantoin, gold, chloroquin, and prednisolone in full dosage, up to 100 mg. daily for 3 months, gave only minimal benefit. She remained febrile and unwell and developed a sacral abscess, severe urinary tract infection and terminal septicemia. She died after a 6 months' illness.

Laboratory findings. These are presented in table 1. The leukocyte count varied from 20,000 to 30,000 cells per cu.mm. throughout the illness. Ten L.E. cell preparations were examined: two were positive, two were “doubtfully positive” in showing suspicious nuclear inclusions, and six were negative. The first clearly positive test was obtained after treatment with penicillin, gold, and corticosteroids; however, one test before any treatment showed rosette formation and nuclear inclusions resembling, but not typical of, L.E. bodies. A liver biopsy showed only slight excess of lymphoid cells in the portal tracts.

Necropsy findings. There were small abscesses in the left kidney, staphylococcal pneumonia, and neoplastic infiltration of the liver, spleen, kidneys and abdominal lymph nodes. The neoplastic tissue consisted of masses of lymphocytes, reticulum cells, eosinophil cells and many conspicuous Sternberg-Reed giant cells; the appearances were those of Hodgkin's disease (fig. 5). Hematoxylin bodies were not detected.

Discussion

Although the clinical features, including severe infection and lymphadenopathy, were compatible with lymphoma in both cases, other findings led to the provisional diagnosis of systemic lupus erythematosus. These included the atypical rash and evidence of hepatic involvement in Case 1, the arthritis resembling early rheumatoid arthritis in Case 2, and positive L.E. cell tests in both cases. However in both there was poor response to corticosteroid treatment, but pronounced improvement with a lymphocytolytic agent (radioactive phosphorus) occurred in Case 1. We considered that in Case 1 the biopsy and necropsy findings established the diagnosis of lymphosarcoma despite the acknowledged difficulty, at times, of distinguishing immunologic and neoplastic proliferations of lymphoid tissue on histo-
logic grounds alone. In Case 2, Hodgkin's disease was discovered at necropsy. It is noteworthy that various autoantibodies, including anticytoplasmic antibody (in weak titer), antithyroglobulin and rheumatoid factor were demonstrated in Case 1 in addition to antinuclear antibody, and the Coombs test was positive in both patients.

There is still no unanimity on the question of the specificity and significance of the L.E. cell phenomenon. Many authorities, cited by Hargraves and Opfell5 and Ogryzlo,6 considered a positive L.E. cell preparation to be pathognomonic of the disease systemic lupus erythematosus (S.L.E.), despite some well-documented clinical evidence to the contrary.7 Especially controversial are certain conditions which differ clinically from S.L.E. and wherein the finding of a positive L.E. cell test occurs with some regularity: these conditions include rheumatoid arthritis, active chronic (lupoid) hepatitis, Sjögren's disease8 and allergic (hypersensitivity) reactions to drugs, particularly penicillin and hydralazine.

Positive L.E. cell tests have been reported previously in single cases of leukemia10 and Hodgkin's disease,11,12 in addition to the present cases; however, on our present experience we would regard the L.E. phenomenon as an exceptional finding in lymphoma and related diseases. Nevertheless the finding, albeit rare, is significant in relation to the “forbidden clone” hypothesis of Burnet.1 Immunologically competent cells are regarded as “forbidden” if they, or the antibodies they secrete, are immunologically reactive with accessible antigens of the host, and the clone from which such cells are derived is called a “forbidden clone.”1 The origin of forbidden clones is presumed to be in somatic mutation or in some equivalent fault in differentiation of lymphoid cells, associated with malfunctioning of a homeostatic process13 which normally suppresses the emergence of these cells of forbidden character.

In a sense, then, the forbidden clones of autoimmune disease may be likened to a conditioned malignancy, being placed at a survival advantage by the availability of the appropriate autoantigen. This concept was also utilized by Dameshek and Schwartz14 in comparing autoimmunization with leukemia, with particular reference to the occurrence of autoimmune hemolytic disease in lymphoid neoplasia. The comparison is further emphasized by histologic resemblances between immunologic and malignant proliferations of lymphoid tissues (vide supra), and by the successful therapeutic use of lymphocytotoxic agents in S.L.E.;15 thus a beneficial effect of such an agent, as in Case 1, might not necessarily differentiate between lymphosarcoma and S.L.E.

If this approach is accepted we are still left in some doubt as to the origin of the multiple autoantibodies in cases of lymphoma, particularly as exemplified in Case 1. The possibilities, which may not be mutually exclusive, are as follows.

(a) The neoplastic cells themselves produce autoantibodies, as exemplified by the antinuclear and other autoantibodies in the present cases, and by hemolytic autoantibodies in hemolytic anemia complicating lymphoid neoplasias.14
The development of lymphoma leads to a “weakness” of homeostatic control in the affected lymphoid tissue, thereby allowing the emergence of other cells carrying forbidden patterns of antibody.

These conditions represent the release of a range of abnormal clones of cells, some with the minimal lack of response to control characteristic of a forbidden clone in Burnet's sense, others covering the range to frank leukemic behavior.

It is interesting to note that the third alternative (c) is very easily fitted into Burch's recent concept of leukemia as dependent on a combination of germinal and somatic mutation.

SUMMARY

Two elderly women suffering from lymphoma—lymphosarcoma and Hodgkin's disease respectively—had positive L.E. cell tests.

In one case the L.E. phenomenon was strongly and consistently positive, as were tests for thyroglobulin antibody and rheumatoid factor; treatment with radioactive phosphorus was beneficial.

The origin of antinuclear and other autoantibodies in lymphoma could be attributed to (a) the development of self-reactivity by the neoplastic lymphoid cells themselves, (b) to weakness of homeostatic control over other self-reactive cells in neoplastic lymphoid tissue, or (c) to the release of a range of abnormal clones, possibly as a consequence of a combination of germinal and somatic mutation.

SUMMARIO IN INTERLINGUA

Duo feminas de etate avantiate qui suifreva de lymphoma—le un e lymphosarcoma e le altere de morbo de Hodgkin—monstrava positivitate in tests pro-cellulas L.E.

In un del casos le phenomeno L.E. esseva fortemente e uniformemente positive, e le mesmo valeva pro tests pro anticorpore thyroglobulinic e pro factor rheumatoidee. Le tractamento con phosphoro radioactive esseva benefic.

Le origine de anticorpores antinucleari e de alte autoanticorpore in lymphoma poteva esser attribuite a (a) le disveloppamento de auto-reactivitate per le neoplastic cellulas lymphoidiee mesme, (b) le debilitate del dominio homeostatic super altre celularis auto-reactive in neoplastic tissu lymphoidiee, o (c) le liberation de un scala de clones anormal, possibilimente in consequentia de un combination de mutation germinal e somatic.

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REFERENCES

3. Gajdusek, D. C.: An 'autoimmune' re-
action against human tissue antigens in certain acute and chronic diseases.

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