HYPOTHESIS

A Case of Multiple Autoimmune Disease, Lymphoid Proliferation and Hypogammaglobulinaemia

By R. E. Sage and I. J. Forbes

Sjögren's Syndrome, originally comprising lymphocytic infiltration and deficient function of the lacrimal and salivary glands,1 has now been found to be associated with many immunologic abnormalities such as antibodies reacting with human tissues and the manifestations of several auto-immune diseases.2,3 Recently, the development of malignant lymphoma in its course has been reported.4

The patient whose case will be described had rheumatoid arthritis and Sjögren's syndrome. She developed autoimmune hemolytic anemia and an unusual lymphocytic response which may have been an early manifestation of lymphoma. Several types of autoantibody were found in the serum, although the total γ-globulin level was low, and analysis of the proteins synthesized by the peripheral lymphocytes showed decreased immunoglobulin production. Treatment with the purine analog azathioprine controlled the hemolytic process after failure of therapy with prednisolone.

A hypothesis, taking into account newer knowledge of the organization of the immunological apparatus, is proposed to explain the coexistence of multiple abnormalities of the lymphoid system, such as autoimmune disease, macroglobulinemia, deficient antibody production, and chronic lymphatic leukemia. The hypothesis is based on the concept of a disorder of terminal immunological differentiation of lymphocytes.

CASE REPORT

A 75 year old Caucasian woman had had rheumatoid arthritis for 21 years, affecting particularly the hands and feet, and had been treated with gold injections and for the past four years with phenylbutazone 50-100 mg. and prednisolone 1.25-2.5 mg. daily. During the past three years, the arthritis had been quiescent. For at least 12 years, there had been episodes of sore, red eyes with a gritty feeling and inability to weep. She was admitted to The Queen Elizabeth Hospital on 24th November, 1964, with a hemoglobin value of 5.8 Gm. percent. She complained of tiredness, severe shortness of breath on exertion, swelling of ankles, nocturnal dyspnoea and throbbing headaches for the past two weeks.
Examination showed signs of left and right ventricular failure. The liver was palpable 7 cm. below the right costal margin and the spleen 12 cm. below the left costal margin. There was no peripheral lymph node enlargement. The thyroid was enlarged 2–3 times normal size, firm and smooth with an enlarged isthmus. Salivary glands were not enlarged. The hands and feet were deformed by rheumatoid arthritis.

Initial investigations showed: Hemoglobin 6.1 gm. percent with spherocytosis, 20 percent reticulocyte count but no Heinz bodies. White cell count was 8,300 per cu. mm. with 55 percent lymphocytes, many of which showed abundant blue cytoplasm, prominent nucleoli and a loose nuclear chromatin pattern (Fig. 1A). Platelet count was 250,000 per cu. mm. The E.S.R. was 100 mm. in the first hour (Westergren method), serum bilirubin 1.5 mg. percent with 1.3 mg. percent indirect reacting, normal thymol and Zinc Sulphate turbidities, alkaline phosphatase 7.7 King Armstrong Units, serum albumin 3.9 gm. percent normal 3.8–5.2), total serum globulin 1.3 gm. percent (normal 1.9–3.1 gm. percent). Cold agglutinins were present to a titer of 1/128 and the direct Coombs’ test was positive to a titer of 1/16.

Sternal marrow examination showed greatly increased cellularity with a normoblastic hyperplasia. The major cellular increase was due to a dense, diffuse infiltration with lymphocytes which comprised 75 percent of the non-erythroid cells present (Fig. 2A). The lymphocytes showed primitive characteristics with an abundant pale blue cytoplasm, usually one and sometimes two prominent nucleoli and a loose nuclear chromatin pattern (Fig. 1B). These findings were thought to be consistent with lymphoma, macroglobulinemia or chronic lymphatic leukemia.

The Hyland Laboratories (Los Angeles, Calif.) latex fixation test for antibody to altered γ-globulin was positive, but the Rose-Waaler test was negative on two occasions. Three examinations for L.E. cells were negative. The autoimmune complement fixation test for antibodies reacting with antigens from thyroid, kidney, lung and stomach were positive to a titer greater than 1/80. Thyroglobulin antibodies were not detected by tanned cell
haemagglutination on two occasions. The half life of Cr$^{51}$ labeled red cells injected on the
tenth day was ten days (normal 26 ± 2 days) with an abnormally high splenic uptake. The
serum electrophoretic pattern showed a low γ-globulin level as well as reduced α and β
globulin peaks.

Schirmer's test and Rose Bengal stain confirmed the presence of kerato-conjunctivitis sicca.

The patient's course is illustrated in Fig. 3. Prednisolone started at 60 mg. orally per day
on the sixth day and later increased to 120 mg. daily on the 12th day resulted in no hematological improvement, but was accompanied by a great increase in the white cell count. This
reached a peak of 26,000 per cu. mm. (18,000 lymphocytes per cu. mm.) on the higher
dosage. The lymphocytes remained abnormal and many smudge cells were seen in the
peripheral blood smears.

Azathioprine 100 mg. per day was given from the 22nd day while the dose of prednisolone
was steadily reduced to 10 mg. per day. During the following week, the hemoglobin rose
slowly to 10 gm. percent, reticulocyte count fell to 10 percent, and white cell count to
10,000 per cu. mm. The liver became palpable and the size of the spleen was reduced
to 5 cms. below the costal margin. The patient was discharged from hospital on the 30th
day.

Several bouts of pneumonia occurred in the next three months in the presence of per-
sistent hypogammaglobulinemia. Respiratory infections were subsequently prevented by
monthly injections of pooled γ-globulin. Each infection was associated with a fall in hae-
oglobin without reticulocytosis (Fig. 3).

Since March 1965, the hemoglobin level has varied between 11.5 and 14.0 gm. percent.
The reticulocyte count remains slightly elevated at 2–4 percent. Spherocytes have not been
present. The white cell count initially fell to 2,500 per cu. mm., but has since slowly re-
turned to normal levels. The abnormal lymphocyte remain in the peripheral blood smears.
The platelet count has varied from 150,000 to 370,000 per cu. mm. The direct and indirect Coombs' tests have become negative.

Cr51 red cell survival in August 1965 showed an improved half life of 17.5 days with the major uptake in the spleen, although this was much less than previously.

Sternal marrow examination at this time showed patchy reduction in cellularity due to a loss of normal erythroid and myeloid elements. The lymphocytes were still abnormal, and were now gathered into follicles as well as being diffusely spread through the marrow (Fig. 2B). Electrophoretic patterns show that the γ-globulin level remains approximately the same. The spleen is still palpable 3–4 cm. below the costal margin.

Serum was tested for autoantibodies in May 1966 by Dr. D. Doniach with the following results:

1. ANF (antinuclear factor test by fluorescence microscopy) positive, titer less than 1/10, with a diffuse fluorescence pattern.
2. No thyroid or gastric parietal cell antibodies were detected by fluorescence microscopy.
3. Smooth muscle fluorescent antibody test was negative.
4. Complement fixation to 1/256 with kidney and stomach homogenate and 1/4 with thyroid homogenate.

**Analysis of immunoglobulin synthesis by the patient's lymphocytes**

Lymphocytes were cultured in the presence of 14C-leucine in February 1966. The soluble proteins from the cells and supernatant were dialyzed after incubation for 24 hours, and were then analysed by autoradiography of the immunoelectrophoretic pattern, by methods...
FIG. 4.—Upper: Immunoelectrophoretic pattern of culture fluid of the patient’s lymphocytes with her own plasma as carrier. The pattern was developed with goat antiserum to IgM and IgA (upper trough) and horse antiserum to whole human serum (lower trough). The strong band nearer to the center of the pattern running parallel to the IgG arc is formed by a precipitin reaction of the goat and horse sera.

Central: Autoradiograph of the upper pattern showing the disposition of the radioactive products. Notice that the IgG, IgA and IgM are labeled only lightly. Lower: Autoradiograph of culture fluid from lymphocytes of a normal subject. The IgG, IgA and IgM lines are much more heavily labeled.

previously described. The autoradiograph is reproduced in Figure 4. The significant features are the reduced labeling of the IgG line, no labeling of IgM, and very weak labeling of IgA.

DISCUSSION

The case history which has been described illustrates the evolution of autoimmune disease over a 21 year period. The first manifestation was rheumatoid arthritis, which was uncomplicated for nine years, when symptoms of Sjögren’s syndrome were first noticed. Keratoconjunctivitis sicca and xerostomia, major manifestations of Sjögren’s syndrome,2,3 developed together with several minor components, including enlargement of the liver and spleen, tracheobronchitis and leukopenia. Hashimoto’s disease and Sjögren’s syndrome are commonly associated.7 Our patient had a goiter, but thyroid antibodies were not detectable in the serum. It is, therefore, unlikely that she had Hashimoto’s disease.8

Twenty-one years after the first symptoms of autoimmune disease were noticed, life-threatening hemolytic anemia developed. The arthritis had then been inactive for three years.

Serological evidence of multiple autoimmune abnormalities accompanied the
clinical manifestations. Antibodies to tissue extracts were detected by the autoimmune complement fixation test. One of the rheumatoid factors was detected by the latex fixation test, but the Rose-Waaler test was negative. The direct Coombs’ test was positive and cold agglutinins were present in slightly raised titer. Antibody to cell nuclei (anti-nuclear factor) was detected by fluorescence microscopy. Contrary to expectation, however, there was a deficit in the circulating immunoglobulins.

The circulating lymphocytes were morphologically abnormal, and the sternal marrow was densely infiltrated by similar cells. These abnormalities could be interpreted as manifestations either of intense immunological activity or of neoplastic lymphoid proliferation, subsequently held in check by azathioprine therapy.

Quantitative measurements of the capacity of this patient’s lymphocytes to synthesize globulin were reported recently, showing a normal basal synthesis of globulin, but no stimulation of synthesis by phytohemagglutinin. Qualitative analyses (Fig. 4) of the protein synthesized showed subnormal synthesis of immunoglobulins.

Fudenberg has recently considered the implications of the relationship between immunologic deficiency, autoimmune disease and lymphoma, which have been observed in the same patient with increasing frequency in the past decade. Autoimmune hemolytic anemia is commonly associated with chronic lymphatic leukemia and lymphomas, sometimes with the paraproteinemias and acquired hypogammaglobulinemia. The last two conditions may occur together in chronic lymphatic leukaeemia, or in association with thymoma. Autoimmune disease is commonly multiple in the one patient. Family studies provide another link between autoimmune diseases, lymphatic leukemia, paraproteinemias and agammaglobulinemia by showing a high incidence of these conditions in relatives of subjects with agammaglobulinemia.

At least three mechanisms have been proposed to explain these syndromes. Through the activity of mutant clones having an antigenic deficiency, which cause a graft-versus-host reaction. The new clones react against the antigen in the cells of the host which they have lost but are not rejected by the host.

2. As a result of a disorder of the thymus, in which clones of cells reacting against self-antigens are not eliminated.

3. Through a genetic defect in lymphocytes in agammaglobulinemia, causing a failure to respond to antigenic challenge, to take part in cellular immune mechanisms and to eliminate clones of lymphocytes with autoimmune activity.

There is a substantial body of evidence, mostly circumstantial or in the nature of analogy with experimental studies, to support hypotheses 1 and 2. Hypothesis 3 is more speculative. Certain objections to it are stated subsequently. The hypothesis to be put forward does not necessarily conflict with 1 and 2. It proposes a mechanism whereby cells with autoimmune activity may arise, and takes into account the effects of malfunction of the thymus.
HYPOTHESIS

It is suggested that the abnormality giving rise to immunologic deficiency, autoimmunity and lymphoproliferative disease in the same patient may be a disturbance of "terminal immunologic differentiation." The hypothesis is based on two major considerations.

1. The common factor in all of these diseases is a multiplicity of abnormalities in the synthesis of immunoglobulins. There may be synthesis of autoantibodies of several specificities, of large amounts of macroglobulin, and deficient synthesis of antibody. In multiple myeloma, macroglobulinemia, and in cases of hypogammaglobulinemia with autoimmune disease, synthesis of abnormal globulin is associated with deficient synthesis of normal globulin. Deficient synthesis of globulin by circulating lymphocytes of patients with macroglobulinemia, chronic lymphatic leukemia and agammaglobulinemia has been demonstrated.

2. Recent evidence points to three stages in the maturation of lymphocytes and the development of immunologic competence:
   (i) stem cells in bone marrow
   (ii) central lymphoid organs
   (iii) peripheral lymphoid organs

   It is postulated that disturbances at any stage may lead to abnormalities of immunological function.

The significance of a disturbance at each level of immunological organization will now be examined with reference to the syndromes exemplified by our case.

A Defect of Bone-Marrow Stem Cells

It is currently thought that the bone marrow contains stem cells giving rise to new lymphocytes in post-natal life. Migration of bone marrow lymphocytes to the thymus has been demonstrated.

Multiple abnormalities of protein synthesis by lymphocytes could conceivably be determined by a genetic defect of the stem cells. It is difficult to reconcile such a notion with our knowledge of the mechanism of protein synthesis. Available evidence indicates that antibody synthesis follows the general pattern of protein synthesis, being directed by DNA-dependent RNA. The structure and specificity of the proteins synthesized by lymphoid cells must therefore be determined by the genetic material of the cells. Accordingly, lymphocytes must possess the genetic code for antibodies of every specificity which the subject is capable of making. The mystery of the immune response is the process of induction of the synthesis of certain specific antibodies while synthesis of the majority is inhibited. It is suggested that this process occurs in peripheral lymphoid organs in response to the presence of antigen.

The process is envisaged as an activation of the relevant part of the genetic code and in these terms multiple abnormalities of antibody synthesis are looked upon as inappropriate activations, or failures to activate the code in the lymphocytes passing through the peripheral lymphoid organs. There is some evi-
Multiple autoimmune disease
dence to suggest that the capacity normally exists to synthesize autoantibodies, but is held in check.28

Multiple abnormalities of protein synthesis may be explained by multiple mutations in the stem cells. However, it seems more likely that a single inherited genetic defect of lymphoid stem cells would cause a single abnormality of immunoglobulin synthesis. Nevertheless, there is insufficient information to exclude the possibility that defects of stem cells may play a part in determining the manifestations of diseases of immunological aberration. Faulty lymphocytes from the bone marrow would presumably not respond normally to maturing and differentiating influences in the thymus and peripheral lymphoid organs respectively.

Derangement at the Level of the Central Lymphoid Organs

The thymus is thought to contribute to the development of immunological competence by stimulating proliferation of lymphocytes within the gland and by secreting hormones which promote immunological competence.29,30 In spite of the fact that a substantial efflux of cells from the thymus has not been demonstrated,31 it seems likely that lymphocytes leave it to enter the body’s pool of small lymphocytes.

Morphological abnormalities support the view that malfunction of the thymus may cause disturbances of function of the lymphocytes responsible for the lesions of autoimmune disease.32 However, thymectomy does not relieve the manifestations of systemic lupus erythematosus33 or prevent autoimmune disease in New Zealand Black mice33,34 in both of which thymic lesions are found. The thymus is normal in many types of immunological deficiency diseases, for example in many cases of acquired agammaglobulinemia, in which rheumatoid arthritis may occur, and in agammaglobulinemia with myeloma.14 On the other hand, some interesting cases of acquired agammaglobulinemia have been described in association with thymoma.15-18 It is possible that lesions of the thymus are a consequence of a derangement originating elsewhere.

Derangement of Terminal Differentiation at the Level of the Peripheral Lymphoid Organs

Lymphocytes are recruited for immunological activity in organs of the third stage, the peripheral lymphoid organs: lymph nodes, spleen, lymphoid collections of the gut and at sites of chronic inflammation. The processing of immunologically uncommitted lymphocytes to react by formation of specific antibody, to become memory cells, to be capable of reacting against a homograft, or to become unreactive to specific antigens (tolerant) has been called terminal differentiation.35 Small lymphocytes pass constantly from blood, through post-capillary venules into the substance of the lymph nodes, and out through the efferent lymphatics.36 When antigen is taken up in a node, small lymphocytes are induced to transform into larger cells, mitose and produce antibody.37,38 The population of lymphocytes leaving the node is then rich in antibody-producing cells.39 This sequence of events is the morphological basis of terminal differentiation. The cellular responses to antigen in both primary and
secondary immune responses occur principally in the lymphoid tissues which take up in the antigen, and do not affect the thymus. Evidence points to a mechanism in the lymph nodes and spleen for controlling immunological activity. For example, the immune response subsides in normal animals after the stimulus has abated—e.g., after infection has been overcome. Just as there is an organization in the spinal cord for deciding the response by the anterior horn cells to a multitude of influences, so there must be an organization in peripheral lymphoid tissue for processing the information impinging on the “final common pathway” of the immunological apparatus. Such influences acting on the final common pathway are antigen, antibody, cortisol, drugs, possibly thymic hormone and estrogen. Macrophages are undoubtedly important cells in this organization. The involvement of macrophages in terminal differentiation is directly supported by the experiments of Fishman. A primary immune response may be initiated in vitro by a soluble extract of macrophages which have been stimulated by antigen. The evidence for a thymic hormone affecting terminal differentiation comes from studies showing the restoration of immunological competence to thymectomized animals by grafts of thymic tissue in cell-impermeable chambers.  

It is clear that there is a disturbance of terminal differentiation in paraproteinemias, chronic lymphatic leukemia and cases similar to ours, as the antibody response to common stimuli is impaired in these conditions. The experiments of Holmes, Gorrie and Burnet with autoimmune hemolytic anemia in New Zealand Black mice demonstrate the existence and subsequent failure of a control mechanism in these animals. They showed that the development of a positive Coombs’ test was conferred on young mice, which had not yet developed autoimmune manifestations, by splenic lymphocytes from older, affected mice. This serological abnormality remitted, only to recur at the time the abnormality usually occurs in these mice. A proportion of the mice develop malignant lymphomas late in the course of their disease. In our hypothesis, development of positive Coombs’ test and autoimmune hemolytic anemia would be regarded as an abnormality of terminal differentiation, an exaggeration of the normal supply of cells reacting against self. Development of chronic lymphatic leukemia would be regarded as total failure of terminal differentiation, with the production of immunologically inert cells. The pleomorphic malignant lymphoma of Hodgkin’s disease may arise in the tissue concerned with directing the terminal differentiation of lymphocytes. The occurrence of autoimmune hemolytic anemia before the development of Hodgkin’s disease may be due to a premalignant lesion in this tissue. A similar sequence of events occurs in mice of the inbred SJL/J strain, which frequently develop a reticulum cell sarcoma resembling Hodgkin’s disease. This is frequently accompanied by a paraproteinemia. Mice with transplanted reticulum cell sarcomas do not have paraproteinemia and may develop profound hypogammaglobulinemia. Sterzl has presented evidence that immunological tolerance is also determined at this level. His experiments showed that in the development of the tolerant state, antibody-synthesizing cells were at first formed but that their
production was subsequently inhibited. Several factors predispose to the development of tolerance: a large dose of antigen, immaturity of the animal, thymectomy in late fetal or early independent life, and depletion of lymphocytes. The tolerant state clearly represents the balance of several factors.

These considerations indicate that disorders of terminal immunologic differentiation are frequently multiple. Our hypothesis proposes that such disorders determine the simultaneous occurrence of chronic lymphocytic leukemia, autoimmune disease, failure of normal immunologic responses and synthesis of paraproteins. No cause or initiating factor has been suggested. We can merely refer to recent studies of Aleutian disease in mink which is similar to multiple myeloma, and autoimmune hemolytic anemia in New Zealand Black mice, which suggest that viruses are involved.

At present, it is impossible to dissociate primary from secondary events in the pathogenesis of these complicated syndromes. Two possible examples of interacting mechanism can be suggested. The emergence of lymphocytes with autoimmune activity against the myo-epithelial cells of the thymus may make a failure of terminal differentiation greater by causing a deficiency of thymic hormone. Virus infection of reticuloendothelial cells may affect bone marrow and thymus as well as peripheral lymphoid tissue. Abnormalities of terminal immunologic differentiation appear to determine the manifestations of these mixed syndromes. This may be a primary event, or secondary to other influences, for example thymic failure, or the presence in the circulation of defective lymphocytes deriving from faulty stem cells or a diseased thymus.

**SUMMARY**

A 75 year old woman who had had rheumatoid arthritis for 21 years and Sjögren’s syndrome for 12 years developed autoimmune hemolytic anemia. Antibodies to tissue extracts, erythrocytes, cell nuclei, and altered γ-globulin were detected in the serum, although the serum γ-globulin level was low.

The bone marrow was densely infiltrated by lymphocytes having similar abnormalities to the circulating lymphocytes.

Analysis of the proteins synthesized by the peripheral lymphocytes showed a relatively low output of immunoglobulins.

The hemolytic process was controlled by azathioprine after unsuccessful treatment with prednisolone.

A hypothesis is put forward to explain the not infrequent association of multiple autoimmune disorders, lymphoproliferative disease and disorders of immunoglobulin synthesis such as hypogammaglobulinemia and paraproteinemia. It is suggested that the disturbance of immunological function in such cases occurs at the site of recruitment for immunological activity in the peripheral lymphoid organs, constituting a disturbance of terminal immunological differentiation. The basic abnormality may, therefore, be found in the supporting cells of the lymphoid organs.

**SUMMARIO IN INTERLINGUA**

Un femina de septanta-cinque annos de etate, con arthritis rheumatoide depost vinti-un annos e syndrome de Sjögren depost dece-duo, disveloppava autoimmun anemia hemolytic.
Anticorpore esseva detegite in le sero con activitate contra extractos tissular, erythrocytos, nucleos cellulari, e alterate globulina γ. Del altere latere, le nivellos seral de globulina γ esseva basse.

Le medulla ossee monstrava un dense infiltration de lymphocytos con anormalitates simile a illos del lymphocytos in le circulation.

Le processo hemolytic esseva maestrate per azathioprina. Un previe essayo therapeutic con prednisolona habeva remanite van.

Es formulate un hypothese que explixarea le non infrequente association de multiplice disordines autoimmun, morbo lymphoproliferative, e disordines del synthese de immunglobulina del typo de hypogammaglobulinemia e paraproteinemia. Es suggestionate que le perturbation del functiones immunologic in tal casos occurre al site del recrutamento pro activitate immunologic in le lymphoide organos peripheric, constituente un disturbation del terminal differentiation immunologic. Le anormalitate fundamental se trova possibilemente, per consequente, in le cellulas supportative del organos lymphoide.

REFERENCES


Hypothesis: A Case of Multiple Autoimmune Disease, Lymphoid Proliferation and Hypogammaglobulinaemia

R. E. SAGE and I. J. FORBES