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Splenomegaly in Northern Nigeria

A. D. M. BRYCESON *, A. F. FLEMING and G. M. EDINGTON

Abstract

Seventy five patients with large spleens were investigated in order to establish the causes of splenomegaly in Northern Nigeria, to define further the diagnostic criteria of tropical splenomegaly syndrome (TSS), and to study its pathogenesis.

Investigations included examination of liver biopsy, bone marrow cytology, lymphocyte response to phytohaemagglutinin (PHA), serum immunoglobulins and complement, and the presence of immunoglobulin and complement fixed in Kupffer cells.

Thirty patients had TSS, five chronic lymphatic leukaemia (CLL), four a syndrome of gross lymphoid hyperplasia (GLH) distinct from TSS, CLL and the lymphomas, and twenty three miscellaneous conventional diseases. In thirteen cases no definite diagnosis could be established.

TSS was found to be predominantly a disease of female Fulani cattle herders. Its essential characteristics were splenomegaly in the presence of acquired immunity to malaria, a grossly raised serum IgM, a lowered serum complement, and the presence of IgM fixed in Kupffer cells. There was lymphoid hyperplasia in bone marrow, hepatic sinusoids and often blood which may be indistinguishable from that in CLL. Lymphocytes undergo normal blastogenesis to PHA. There was clinical and haematological response to proguanil therapy. Reticulo-endothelial phagocytosis of IgM, probably as a complex, seems to be the essential feature of the condition. As it was impossible to identify early cases of TSS it is unclear whether IgM overproduction or phagocytosis of IgM complexes is the first stage of the disease. The precise nature of the association with malaria remains obscure.

The diagnosis of CLL demanded the demonstration of an abnormally low immunoglobulin level and impaired lymphocyte responsiveness to PHA by blast transformation or ³H-thymidine incorporation, in addition to the usual haematological findings.

The syndrome GLH occurred in multiparous Hausa women. It was characterised by intense lymphocytosis with active, PHA-responsive cells, and normal immunoglobulin levels. Patients responded to proguanil therapy. It is suggested that these patients have a depressed immune response to malaria, perhaps through repeated pregnancies, and to a leukaemogenic agent, both of which stimulate lymphocytosis. Antimalarial treatment at this stage may prevent the development of frank leukaemia or lymphoma.

The usefulness of the various investigative procedures and the problem of managing the large number of undiagnosed cases are discussed.

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Introduction

Out of the miscellany of splenomegaly of unknown origin in the tropics there has emerged in the past ten years a syndrome characterised by:

1. Gross splenomegaly and moderate hepatomegaly in the presence of acquired immunity to malaria (PRYOR, 1967a; WATSON-WILLIAMS & ALLAN, 1968).
2. Hypersplenism as shown by red cell pooling and haemolysis, granulocytopenia, thrombocytopenia and plasma volume expansion (RICHMOND, DONALDSON, WILLIAMS, HAMILTON & HUTT, 1967; PRYOR, 1967b).
3. Kupffer cell hyperplasia and lymphocytic infiltration of hepatic sinusoids (MARSDEN, HUTT, WILKS, VOLLER, BLACKMAN, SHAH, CONNOR, HAMILTON, BANWELL & LUNN, 1965).
4. Other features of lymphocytic proliferation, such as peripheral lymphocytosis and infiltration of bone marrow with mature lymphocytes (WATSON-WILLIAMS & ALLAN, 1968; SAGOE, 1970).
5. Normal lymphocyte function as judged by blastic transformation in the presence of phytohaemagglutinin (PHA) (ZIEGLER, COHEN & HUTT, 1969; SAGOE, 1970).
6. Grossly normal induction and expression of humoral and cellular immune responses to antigenic challenge (ZIEGLER, COHEN & HUTT, 1969; CRANE, ROWLEY, WARBURTON & MACKAY, 1972).
7. Abnormally high levels of serum IgM and the presence of circulating macroglobulins containing IgM (WELLS, 1970; ZIEGLER, 1973).
8. An association with malaria suggested epidemiologically (HAMILTON, MORROW, ZIEGLER, PIKE, WOOD, BANYIKIDDE & HUTT, 1969), by high titres of anti-malarial antibody (GEBBIE, HAMILTON, HUTT, MARSDEN, VOLLER & WILKS, 1964) and by response to antimalarial therapy (WATSON-WILLIAMS & ALLAN, 1968; SAGOE, 1970) and by partial protection from the condition in those with sickle cell trait (HAMILTON, MORROW, ZIEGLER, PIKE, WOOD, BANYIKIDDE & HUTT, 1969).
9. Possible racial and familial predispositions (HAMILTON, HUTT, WILKS, OLWENY, NDAWULA & MWANGE, 1965; ZIEGLER & STUIVER, 1972).

The name tropical splenomegaly syndrome (TSS) was suggested for this condition by PITNEY (1968).

When the haematology clinic was opened in Zaria in 1971, it became clear that splenomegaly was a common complaint among adult patients. We decided to investigate these patients in order to:

1. establish the relative frequency of different causes of splenomegaly in adults in northern Nigeria;
2. define diagnostic criteria for TSS against chronic lymphatic leukaemia (CLL), lymphomas and other causes of massive lymphoid hyperplasia;
3. study the pathogenesis of TSS.

Patients and Methods

One hundred consecutive patients over the age of seven years and referred on account of splenomegaly were admitted to the study; it was our intention to include all patients who would normally have acquired immunity to malaria during childhood, and who had spleens greater than 6 cm below the costal margin. Investigations were incomplete in twenty four patients; and one patient was found at laparotomy to have a retroperitoneal tumour, not an enlarged spleen. The results in the remaining seventy-five patients were analysed. Of the patients whose homes could be identified on a map, 40% were local, 30% came from ten to one hundred miles away, 8% from over one hundred miles and 22% were nomadic Fulani cattle herdsman and women coming from any distance up

to several hundred miles. Three quarters of the long distance patients came from north of Zaria.

The size of the spleen was measured from its tip to the costal margin along its longest axis. The liver was measured from its edge to the costal margin in the midclavicular line.

The following investigations were performed by standard methods: haemoglobin concentration (Hb), packed cell volume (PCV), red cell appearance, total and differential leucocyte count, platelet count, malaria parasites on single thin blood films, bone marrow aspiration from the anterior iliac crest, Hb electrophoresis, total serum proteins and serum electrophoresis, serum glutamic oxaloacetic and pyruvic transaminases and brucella agglutinins. Stools were examined for helminth ova by simple smears and flotation concentration techniques. Rectal snips were examined for schistosome ova.

Serum immunoglobulins and complement (C3) were measured by the Mancini technique (FAHEY & MCKELVEY, 1965) using monospecific antisera (Hyland Laboratories) and calibrated against World Health Organisation standard sera kindly supplied by Dr. D. S. Rowe. The distribution of immunoglobulin values was skew, but there was a normal distribution of logarithmic values: the means were expressed as the antilog of ($\Sigma \log x$) and the 95% confidence limits as the antilog of ($\log \text{mean} \pm 2 \log \text{SD}$). The complement standard was our own pool of local sera, which was divided into aliquots, freeze-dried and stored at -40°C . Lymphocytes were cultured in 15% autologous serum/199 medium (Borroughs Wellcome Laboratories) for seventy two hours with or without 0.1 ml 5% reconstituted PHA (Borroughs Wellcome). Two microcuries ^3H thymidine (Radiochemicals, Amersham) were added eighteen hours before harvest. Blastic transformation was assessed both by beta emission, which was expressed as the ratio of disintegrations per minute in PHA stimulated cultures compared with unstimulated cultures, and also directly as percentage counts of lymphoblasts in cytocentrifuged preparations stained with May-Grunwald-Geimsa stain.

Percutaneous liver biopsy specimens were fixed in 10% neutral formol saline, embedded in paraffin and stained with haematoxylin and eosin. The following stains were used when indicated; Perl's for iron, Ziehl-Neelsen for acid fast bacilli, periodic acid-Schiff and Schmorl for lipofuscin, Gmelins reaction for bile and a silver reticulin stain. Surgical biopsy of lymph nodes was performed when necessary and diagnostic laparotomy was carried out on four patients.

A diagnosis of TSS was considered likely in patients who had no other cause for splenomegaly and in whom at least three of the four following criteria were met:

1. The serum IgM was above the local rural mean + 2 SD (762 IU) per ml).
2. Hepatic sinusoidal infiltration was present.
3. Over 60% of lymphocytes responded to PHA *in vitro*.
4. There was a progressive reduction in spleen size accompanied by haematological and subjective improvement while on continuous proguanil treatment.

The diagnosis of TSS was usually considered excluded if there was another cause for splenomegaly, the serum IgM was below the control mean, under 40% lymphocytes responded to PHA or there was no improvement on continuous proguanil treatment. These criteria were in part established retrospectively and in many instances no firm initial diagnosis was made.

The diagnosis of CLL required not only gross lymphocytosis of peripheral blood and bone marrow but in addition features which denied a diagnosis of TSS.

All patients were treated with chloroquine 600 mg (base) statim and 300 mg twice a day for two days, followed by proguanil 100 mg daily indefinitely and with other treatment as indicated. Patients were reassessed every three months.

Results

Definite diagnosis was reached in fifty-eight of the seventy-five patients (Table I). There was a group of four patients who were neither TSS nor CLL, but were characterised by gross lymphoid hyperplasia (GLH) of unknown origin. There were thirteen patients in whom no definite diagnosis (NDD) could be made. Twenty-three patients with miscellaneous diagnoses listed in Table I are not described further.

Sex

Females predominated in the ratio of 2:1, except in the NDD group (Table I). This predominance was not found under the age of twenty, although the total number was too small for this to be significant. The female: male ratio of medical admissions in Zaria is 1:1.4.

Tribe

Hausa is the main tribe around Zaria, and the second tribe, the Fulani, accounts for only 3% of all hospital medical admission. In the TSS group Fulanis predominated 3:2, while Hausas and other tribes predominated 5:1 in all other groups ($P < 0.01$). Fifteen of the eighteen Fulani with TSS were nomadic cattle herders, who came to hospital as they passed Zaria on their annual migration. No cases of CLL, GLH or other lymphoreticular or haemopoietic tumours were seen among the Fulani.

Age

Patients with TSS ranged from ten years to over sixty, but half were in their third and fourth decades (Table II).

Hepatosplenomegaly and lymphadenopathy

Spleen size ranged from 6–38 cm (Table III). The size of the spleen was no indication of the cause of its enlargement. The liver was palpable in twenty-six of thirty-one cases of TSS but was under 10 cm in all but two patients. The largest liver, 21 cm, was in a GLH patient.

Generalised lymphadenopathy was moderate in five TSS, one CLL, three GLH, four NDD and gross in one each TSS, CLL and NDD.

Malaria, haemoglobin electrophoresis and anaemia

P. malariae was seen on thin blood films of two Hausa patients with TSS. *P. falciparum* was seen in two patients, one each with CLL and

Table I. Diagnosis in 75 Patients with Splenomegaly

| | Male | Female | Total | % |
|--|------|--------|-------|-----|
| Tropical splenomegaly syndrome (TSS) | 10 | 20 | 30 | 40 |
| Chronic lymphatic leukaemia (CLL) | 2 | 3 | 5 | 7 |
| Gross lymphoid hyperplasia (GLH – not TSS or CLL) | 0 | 4 | 4 | 5 |
| No definite diagnosis (NDD) | 6 | 7 | 13 | 17 |
| Miscellaneous diagnosis* | 9 | 14 | 23 | 31 |
| | 27 | 48 | 75 | 100 |

* Chronic myeloid leukaemia (3), acute leukaemia (2), lymphosarcoma (3), sickle cell disease (Hb. SS) (5), iron deficiency (1), tuberculosis (2), brucellosis (1), malaria in a semi-immune (1), hepatic cirrhosis with portal hypertension (3), portal hypertension without cirrhosis (2).

Table II. Age of Patients with Splenomegaly

| Years | TSS (30) | CLL (5) | GLH (4) | NDD (13) |
|-------|----------|---------|---------|----------|
| <20 | 9 | 0 | 0 | 2 |
| 20–29 | 4 | 1 | 1 | 3 |
| 30–39 | 8 | 1 | 1 | 4 |
| 40–49 | 6 | 2 | 2 | 2 |
| 50–59 | 2 | 1 | 0 | 1 |
| 60–69 | 1 | 0 | 0 | 1 |

Table III. Size of Spleen in Patients with Splenomegaly

| Spleen size (cm) | TSS (30) | CLL (5) | GLH (4) | NDD (13) |
|------------------|----------|---------|---------|----------|
| <10 | 4 | 0 | 0 | 3 |
| 10–19 | 19 | 5 | 1 | 5 |
| 20–29 | 6 | 0 | 2 | 4 |
| 30+ | 1 | 0 | 1 | 1 |

NDD, in whom severe immuno-depression was suggested by impaired PHA responses and low immunoglobulin levels.

Hb electrophoresis was performed in forty patients. Hb AS was seen in two of twenty-two patients with TSS and four patients in the other groups. The expected numbers would be six in each category,

Table IV. Total white cell count, neutrophil polymorphs, lymphocytes and platelets in 123 Zaria blood donors and 30 patients with TSS

| | Blood donors | | TSS | | P | | |
|-------------------------------|---------------|--------------|---------------|------|-----------------|-----|----------|
| | Mean \pm SD | | Mean \pm SD | | | | |
| Total white cells per μ l | 5492 \pm | 2061 | 7413 \pm | 7357 | 0.02 > P > 0.01 | | |
| Neutrophiles per μ l | 3154 \pm | 1550 | 2614 \pm | 1815 | NS | | |
| Lymphocytes per μ l | 1910 \pm | 882 | 4485 \pm | 7010 | P < 0.01 | | |
| Platelets per μ l | 199 | 990 \pm 96 | 660 | 122 | 571 \pm 71 | 840 | P < 0.01 |

but the figures were too small to reach significance. Two patients in group NDD had Hb AC.

Hb values among Hausa blood donors were mean 12.6 g per 100 ml \pm 1.2 g (SD) (ATANU, OMEJE & FLEMING, 1974) and among Fulani herders 12.5 g per 100 ml \pm 2.3 g (SD) (LEEFLAND, P., personal communication). In patients with TSS the mean was 8.7 g per 100 ml \pm 2.0 g (SD). In CLL and GLH mean values were 7.2 g per 100 ml and 7.4 g per 100 ml respectively. Mean values for the three groups differed significantly ($P < 0.01$) from the controls, but not from each other. However, individual values below 6.7 g per 100 ml were seen in only one patient with TSS, two each of CLL and GLH and on one NDD.

The anaemia in TSS was normocytic and normochromic unless complicated by iron or folate deficiency (Table VI).

Peripheral blood counts

The mean total white cell count in TSS was significantly higher than that of blood donors (Table IV). Seventeen out of thirty TSS patients had more than 50% lymphocytes in the differential count; this lymphocytosis was both relative, due to neutropenia, and absolute.

Lymphocyte counts of twenty four TSS patients fell within the 90% confidence limits of the blood donors, but the mean count was significantly higher (Table IV). Two patients had a lymphocytosis so high as to suggest a diagnosis of CLL; lymphocyte counts less than 1,000 per μ l were seen in three patients with uncertain diagnoses, but in only one TSS patient (Table V).

The range of neutrophil polymorph counts in blood donors was 1,170 to 6,390 per μ l. This tendency to neutropenia was more pronounced in TSS (Table IV); four patients had counts of 1,000 per μ l or less.

Table V. Absolute Lymphocyte Counts in Peripheral Blood of Patients with Splenomegaly

| Lymphocytes per μ l | TSS (30) | CLL (5) | GLH (4) | NDD (13) |
|-------------------------|----------|---------|---------|----------|
| <1,000 | 1 | 0 | 0 | 3 |
| 1,000–4,000 | 24 | 0 | 1 | 6 |
| 4,000–10,000 | 3 | 0 | 0 | 1 |
| 11,100–20,000 | 0 | 1 | 0 | 3 |
| 21,100–30,000 | 1 | 0 | 0 | 0 |
| 31,100–40,000 | 1 | 1 | 1 | 0 |
| 41,000+ | 0 | 3 | 2 | 0 |

Eosinophilia was not a feature of TSS. Two patients had pronounced eosinophilia; one patient with *D. perstans* infection had 744 eosinophils per μ l (12%) and one with heavy hookworm infestation had 1,035 per μ l (23%).

The platelet counts in blood donors were in the range of 68,000–370,000 per μ l; patients with TSS showed significant thrombocytopenia (Table IV) and ten had counts less than 68,000 per μ l, the lowest being 39,000 per μ l. No patient showed signs of haemorrhage or purpura.

Bone marrow cytology

An excessive infiltration with lymphocytes was a characteristic common to TSS, CLL and GLH (Table VI). It was seen in fourteen patients with TSS and was so gross in five as to suggest a diagnosis of CLL. The lymphocytes were usually mature small cells, but were pleomorphic in one patient each with TSS, GLH and NDD. Cultured lymphocytes of these patients showed high spontaneous blast transformation (*vide infra*).

In the group NDD an excessive infiltration with normal lymphocytes failed to correspond in any way with immunoglobulin levels, liver biopsy findings or lymphocyte response to PHA (*vide infra*).

One third of patients with TSS had no stainable iron in the bone marrow. These patients were all Fulanis, and half had hookworm infection. One third of patients with TSS and one half with NDD had megaloblastic erythropoiesis.

Lymphocyte response in vitro to phytohaemagglutinin (PHA)

In unstimulated, control, cultures (Figure 1), blast counts of over 10% were noted in ten patients (four TSS, three NDD – but none with

Table VI. Summary of Bone Marrow Cytology in Patients with Splenomegaly

| Characteristic | TSS (29) | CLL (5) | GLH (4) | NDD (13) |
|----------------------------|----------|---------|---------|----------|
| Megaloblastic | 9 | 0 | 0 | 6 |
| Granulocytic proliferation | 2 | 0 | 0 | 2 |
| Excess eosinophils | 3 | 0 | 0 | 2 |
| Excess lymphocytes | 14 | 5 | 3 | 5 |
| Iron deficiency | 10 | 0 | 0 | 1 |

CLL). The greatest unstimulated response (87%) was in a patient who may have had tuberculosis. These high background blast counts and the frequent presence of large immature lymphocytes in culture made it extremely difficult to assess accurately the extent of transformation to PHA in several instances, especially in the group GLH.

As assessed by blast counts, lymphocytes either did (> 60% blasts) or did not (< 40% blasts) respond normally to PHA.

Blast transformation was normal in all but one patient with TSS; she was a Fulani woman with hookworm, iron deficiency, chronic urinary tract infection, toxic granulation of neutrophils and an excess of plasma cells in the marrow. As all other criteria of TSS were met, it was thought that her intercurrent illnesses may temporarily have suppressed lymphocyte responsiveness.

Transformation was normal in two and depressed in three patients with CLL. Patients with GLH responded better than those with CLL, only one of four being depressed. Patients with NDD were clearly divided into ten responders and three non-responders. The poor response in one patient may be accounted to chicken pox, which he developed two days later, but no cause was apparent in the other two.

Lymphocyte transformation assessed by thymidine incorporation corresponded poorly with blast counts and only at the extremes of the ranges (Figure 1), whether recorded as incremental disintegrations/unit time or as the ratio of incorporation between stimulated and unstimulated cultures. This was especially noticeable in patients with TSS. Low blast counts in the other groups did tend to correlate with low ratios, but the inverse was not true. Patients with CLL all had ratios under thirty, despite high blast counts in two. The ratios were all high in GLH, suggesting a fundamental difference in lymphocyte function between these two groups.

Liver histology

Hepatic sinusoidal lymphocytosis was graded according to the criteria of MARS DEN, CONNOR, VOLLER, KELLY, SCHOFIELD & HUTT

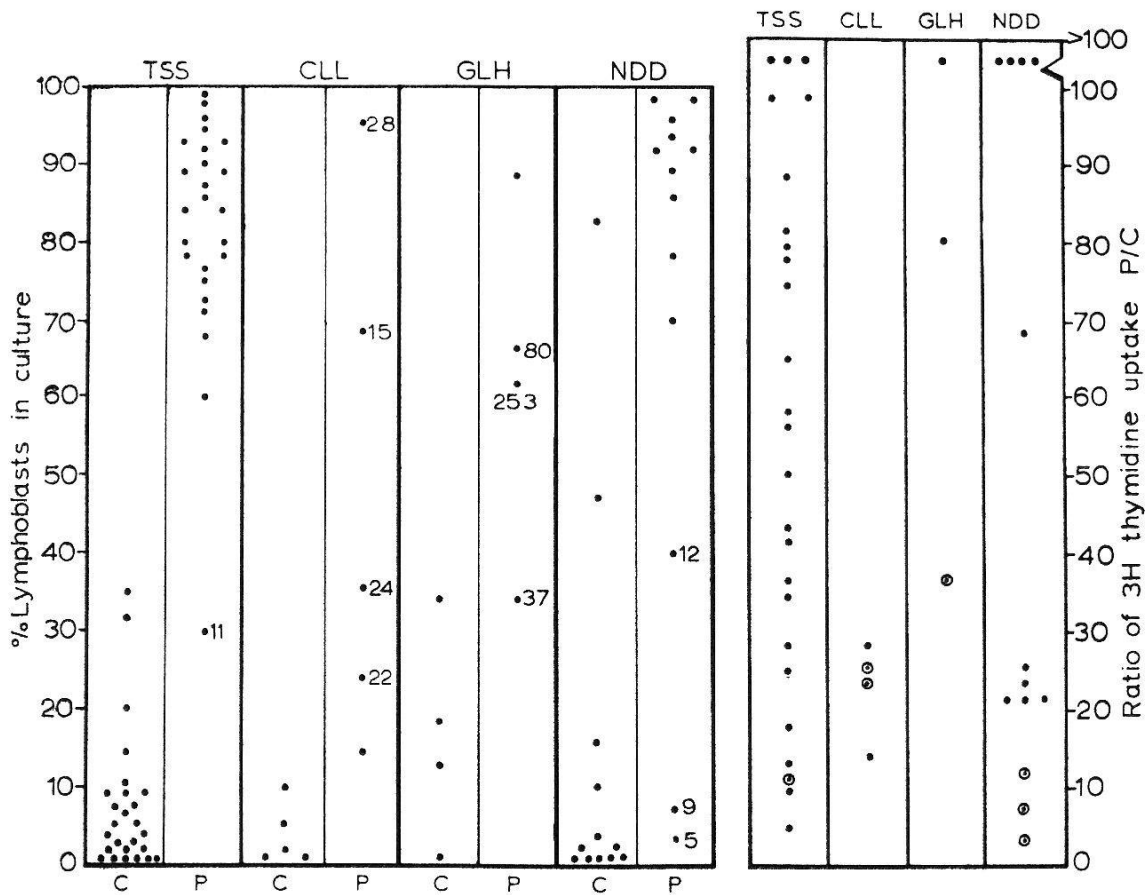


Fig. 1. Lymphocyte responses, *in vitro*, to phytohaemagglutinin in four groups of patients with splenomegaly, as assessed by lymphoblastic transformation and by 3H-thymidine incorporation. Abbreviations. TSS=tropical splenomegaly syndrome, CLL=chronic lymphatic leukaemia, GLH=gross lymphoid hyperplasia, NDD=no definite diagnosis, C=control unstimulated culture, P=culture stimulated with phytohaemagglutinin. Numbers on the left hand plot indicate the corresponding ratio value of 3H thymidine uptake. Ringed dots in the right hand chart indicate less than 40 per cent blast transformation.

Table VII. Summary of Liver Biopsy Findings in Patients with Splenomegaly

| | TSS (30) | CLL (3) | GLH (3) | NDD (13) |
|---|----------|---------|---------|----------|
| H.S.L.* Grade 0 | 5 | 1 | - | 3 |
| 1 | 10 | - | 1 | 5 |
| 2 | 5 | 1 | - | 2 |
| 3 | 10 | 1 | 2 | 3 |
| Hypertrophied Kupffer cells | 30 | | 3 | 9 |
| Iron pigment | 4 | - | - | 2 |
| Malarial pigment | 6/28 | 1 | 1 | 10 |
| Severe cellular infiltration of portal tracts | 4 | 2 | 3 | 7 |

* H. S. L. Hepatic sinusoidal lymphocytosis.

Table VIII. Immunoglobulin Values in Normal Subjects and in Patients with Splenomegaly

| Groups | IgM IU/ml | | IgG IU/ml | | IgA IU/ml | |
|------------------------------|-----------|-----------|-----------|----------|-----------|--------|
| | Mean | 95% CL | Mean | 95% CL | Mean | 95% CL |
| Hausa farmers (100) | 332 | 203- 541 | 210 | 121- 399 | 84 | 12-257 |
| Fulani herders (71) | 359 | 128- 999 | 262 | 65-1055 | 95 | 31-288 |
| Significance | NS | | P<0.01 | | NS | |
| Pooled rural controls (171) | 343 | 161- 762 | 230 | 81- 653 | 88 | 29-274 |
| TSS (30) | 2143 | 317-14150 | 233 | 100- 547 | 48 | 14-161 |
| Significance vs. pool | P<0.01 | | NS | | P<0.01 | |
| CLL (5) | 147 | 21- 1021 | 113 | 59- 213 | 49 | 15-158 |
| Significance vs. pool | P<0.01 | | P<0.01 | | P<0.01 | |
| Significance vs. TSS | P<0.01 | | P<0.01 | | NS | |
| GLH (4) | 122 | 13- 1079 | 265 | 121- 583 | 63 | 13-288 |
| Significance vs. pool or TSS | P<0.01 | | NS | | NS | |
| Significance vs. CLL | NS | | P<0.01 | | NS | |

NS = not significant.

95% CL = 95% confidence limits (see text).

(1967). It was present in twenty-five of thirty patients with TSS and in ten with NDD, in all of whom a confident liver biopsy diagnosis of TSS was made (Table VII). It was also seen in two patients each with sickle cell disease and lymphosarcoma, and one each with portal hypertension without cirrhosis and malaria in a semi-immune adolescent. Five cases of TSS did not have hepatic sinusoidal lymphocytosis, but were typical in every other respect.

Hypertrophied and hyperplastic Kupffer cells were present in all cases of TSS, but this finding was not helpful in diagnosis as these appearances were also common in the other liver biopsies (Table VII). The cells were strap like, or rounded and the cytoplasm was palely eosinophilic with at times indefinite or irregular cell borders. Erythrophagocytosis was not noted. Small amounts of iron pigment were noted in four biopsies only. Haemozoin of malarial or schistosomal origin was present in six of twenty eight TSS liver biopsies but in only one instance were heavy deposits noted in the Kupffer cells and portal tracts, and this was in a woman suffering from a severe *P. malariae* infection. By contrast, haemazoin was present in ten of thirteen cases of NDD. In an unselected series of one hundred and one adult liver biopsies in Zaria, haemozoin was noted in 48% of males and 50%

of females (EDINGTON, in preparation). Few of these biopsies showed evidence of schistosomal infection and it was concluded that the haemozoin was malarial. The relative absence of malarial pigment in TSS and the relative increase in NDD were both significant ($P < 0.01$).

Severe cellular infiltration of portal tracts appears to be less common in TSS than in CLL, GLH and NDD. The cells were mainly lymphocytes and monocytes with a few plasma cells. The occasional presence of eosinophils aroused suspicion of schistosomiasis. A schistosomal granuloma, however, was only seen in one patient with TSS and one with GLH. In these two cases only were ova of *S. mansoni* present in the stool and rectal snip.

A mild degree of liver cell dysplasia and occasional focal necroses were seen in TSS, but no more commonly than in the unselected controls.

Immunoglobulins

Immunoglobulin values were estimated in two rural populations in northern Nigeria, one hundred rural Hausa farmers and their families from the area of Malumfashi about eighty miles north of Zaria (MOHAMMED, TOMKINS & GREENWOOD, 1973) and seventy-one nomadic Fulani cattle herders (unpublished) (Table VIII). Separate values for the two groups show slight differences in mean IgM and IgG values, the latter being significant. The pool contained one hundred and sixteen males and fifty-four females, the age range being five to fifty-five years. There was therefore a close fit for age, sex, tribe and area of habitation with the patients with splenomegaly. The calculated 95% confidence limits for IgM corresponded closely with the observed fifth and ninety-fifth centiles (170 and 860 IU per ml).

TSS: The mean of IgM was markedly higher in TSS than in the controls ($P < 0.01$) (Figure 2, Table VIII); twenty-eight of thirty patients had IgM values above the control 95% confidence limits and the remaining two values were above the normal mean. Values were over ten times the control mean in one third of TSS patients. IgM levels did not correlate with spleen size. IgG levels were normal, but IgA levels were significantly low with nine of thirty patients having values below the normal range.

CLL: Mean values were significantly low for all three immunoglobulin classes (Table VIII). Only two values in fifteen were above the control mean. The top IgM value overlapped with the lowest value in TSS.

GLH: The mean value for IgM was significantly low (Table VIII). One of four individual values was abnormally low, but all IgG values were normal.

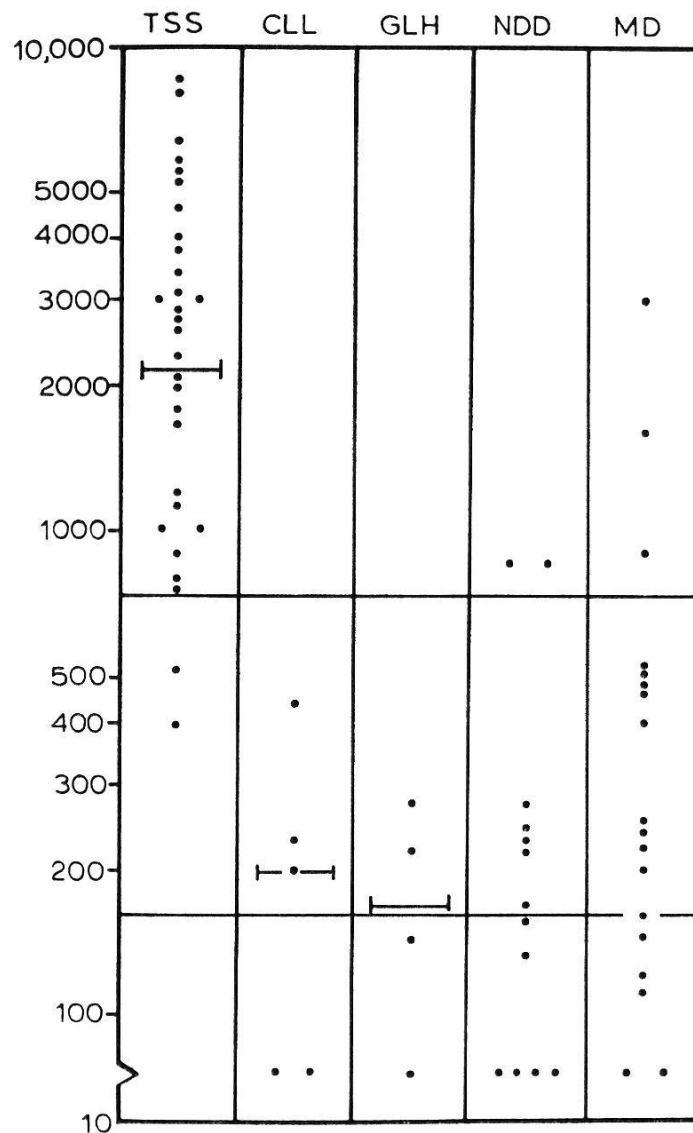


Fig. 2. Serum IgM values, expressed in International Units, of five groups of patients with splenomegaly (see Figure 1 for abbreviations, MD = miscellaneous diagnoses; Table I). —| indicates mean value. Horizontal lines indicate 95 per cent confidence limits of normal values (Table VIII).

NDD: All three immunoglobulin levels were low in one patient (Number 92, Table XII), who may have had a lymphoma. IgM levels were low in five and IgG in one other patient.

Serum complement

Complement levels in TSS were significantly lower than normal (Table IX) and sixteen of twenty-five patients had values below 70% compared with two of twenty one in all the other groups. There was an inverse correlation between complement levels and IgM of patients with TSS ($r = -0.603$) (Figure 3). The ratio IgM/C3 was above ten in

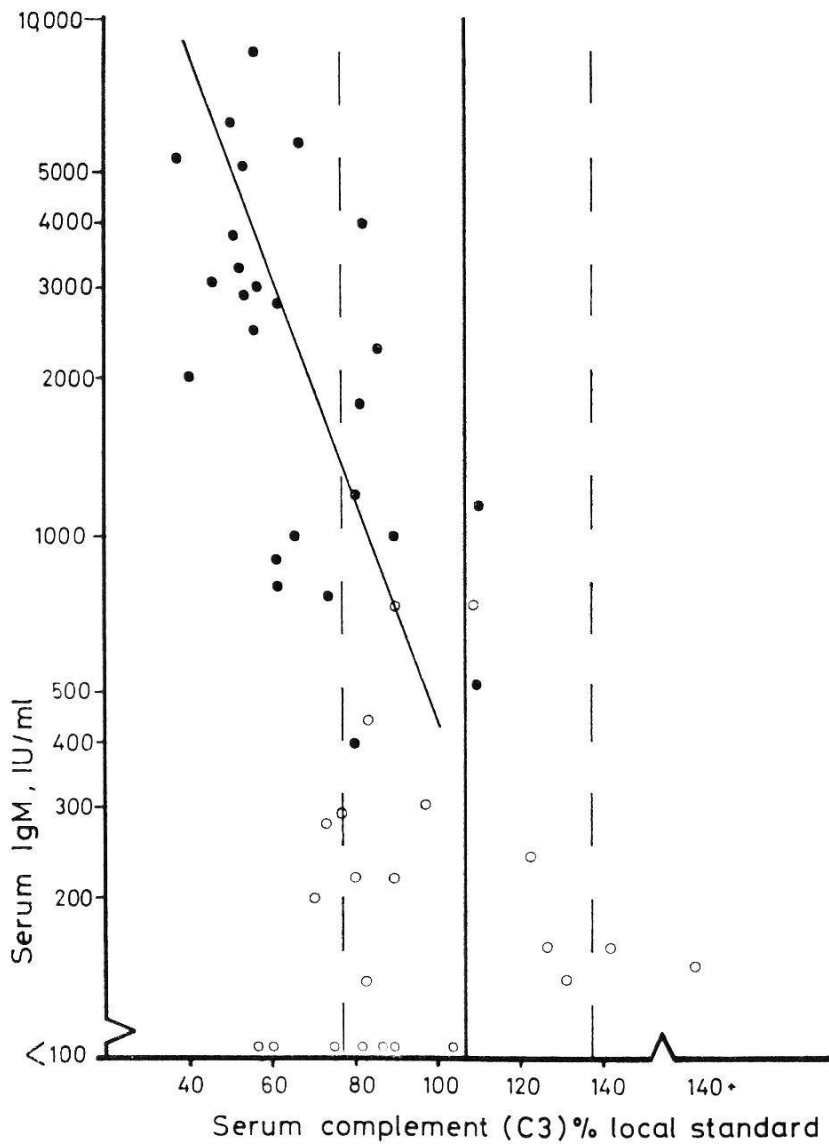


Fig. 3. Relationship between serum IgM and complement levels in patients with tropical splenomegaly syndrome ●, and in those in all other groups ○.

Table IX. Serum Complement Values in Control Subjects and Patients with Splenomegaly

| | Controls (84) | TSS (24) | CLL (5) | GLH (4) | NDD (13) |
|---|---------------|----------|---------|---------|----------|
| Mean + SD | 107 ± 30 | 66 ± 19 | 88 ± 27 | 77 ± 5 | 93 ± 22 |
| Significance of difference from control | — | P < 0.01 | NS | NS | NS |

Results are expressed as a percentage of our own reference Zaria standard of freeze dried pooled adult sera. The control subjects are Hausa farmers and their families over the age of 11 years.

twenty-two of twenty-five patients with TSS and below four in sixteen of twenty-two patients in all the other groups. However, when IgM levels in TSS were below about 1,000 IU per ml, complement levels were not consistently low and the IgM/C3 ratio did not clearly separate TSS from the other conditions. In other groups complement levels were normal and showed no relationship with IgM.

Immunoglobulins fixed in Kupffer cells

Twenty-two liver biopsies were examined for the presence of IgM, IgG, IgA and complement by immunofluorescence (Table X). Eleven of thirteen TSS patients had IgM fixed in Kupffer cells as compared with two of nine of the others ($X^2 = 6.178$; $P < 0.01$). One of the two negative TSS patients (patient 64, Table X), a Fulani female, may represent the normal upper limit of splenic hypertrophy and IgM response to unstable malaria; the other, a Hausa female, had a heavy *P. malaria* infection and it is conceivable she was suffering from chronic malaria rather than TSS. One of the two positives among the others was classified as GLH (patient 79, Table XI) and the other, a Fulani male with IgM of 900 IU per ml and serum complement of 60% could possibly have had TSS in association with non-cirrhotic portal hypertension.

There also seemed to be a negative correlation between IgM and haemozoin in Kupffer cells. In livers whose Kupffer cells contained IgM, only one of thirteen contained pigment as compared with five of eight livers not containing IgM, but this difference did not reach significance ($X^2 = 4.85$; $P > 0.05$). It was noticed that haemozoin in Kupffer cells of frozen liver biopsies gave a characteristic red fluorescence using KP 300 exciter filter and a barrier filter of 470 nm. It was thus possible to look for haemozoin and IgM on the same section. The detection of haemozoin by this method agreed in 14/16 cases with light microscopy findings. It was not, however, possible to distinguish malarial from schistosomal pigment. One patient only in each group had proven schistosomiasis *mansoni*.

Response to treatment

The patients felt better, the spleen size shrank, IgM levels fell, and Hb rose in all twelve cases of TSS where proguanil was taken regularly and the patient returned for follow up. IgM levels fell by 280–3,300 IU per ml. Improvement was detectable within three months of starting proguanil and continued steadily, but at varying rates (Figure 4). Two

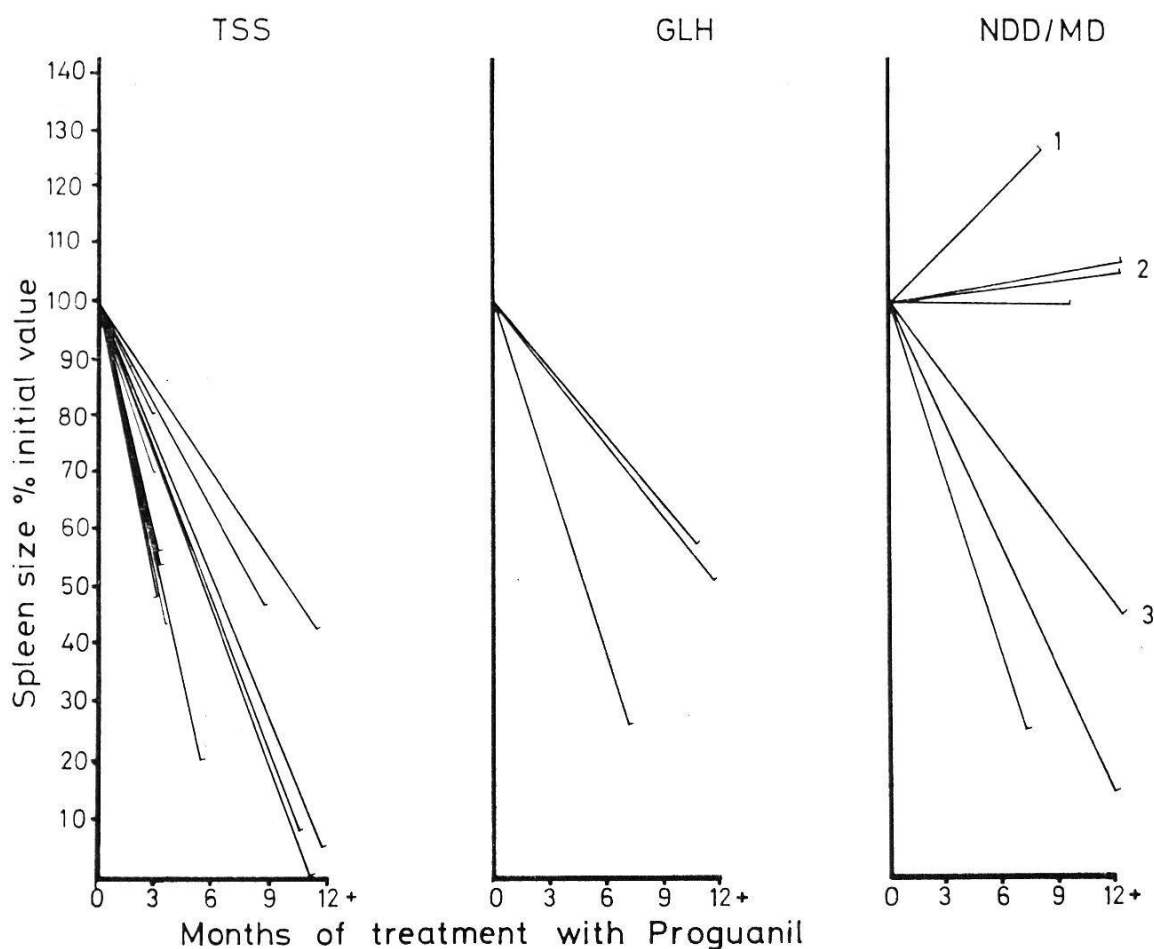


Fig. 4. Reduction in spleen size during continuous treatment with proguanil in four groups of patients (see Figures 1 and 2 for abbreviations).

patients who stopped taking proguanil were retraced and the spleens had increased in size.

Proguanil treatment was associated with clinical improvement, rising Hb and marked reduction in spleen size in three patients with GLH (Table XI), two with NDD and one with lymphosarcoma in whom the final diagnosis was not made for fifteen months (Figure 4). However, IgM levels, which were not raised initially, did not change appreciably. In one GLH patient (number 79, Table XI) the picture was complicated by an eight week course of cyclophosphamide.

One patient with NDD, tentatively called early TSS (Table XII) took proguanil for three months only and the spleen shrank from 22 cm to 3 cm. When seen three years later, the spleen had not enlarged again,

Follow up was extremely difficult and disappointing. Seventeen patients diagnosed as TSS were never seen again: sixteen of these were nomadic Fulanis, a group who seldom seek medical aid and whose medical problems are largely unknown. Forty other defaulters were thought to be settled at the addresses they had given, but only seven could be traced during a three week search.

Table X. Immunoglobulins and Complement in Kupffer Cells

| Series No. | Diagnosis | IgM | IgG | IgA | C3 | Pigment in K cells | HSL | Serum IgM (IU/ml) | Spleen cm | Notes |
|------------|-----------|-----|-----|-----|----|--------------------|-----|-------------------|-----------|---------------------|
| 47 | TSS | + | + | - | - | - | ++ | 8000 | 14 | - |
| 64 | TSS | - | - | - | - | - | - | 2300 | 8 | - |
| 67 | TSS | +++ | - | - | - | - | ++ | 8500 | 23 | - |
| 72 | TSS | +++ | - | - | - | - | + | 900 | 13 | <i>S. mansoni</i> |
| 73 | TSS | - | - | - | - | - | ++ | 1680 | 12 | <i>P. malariae</i> |
| 75 | TSS | +++ | - | - | - | - | ++ | 1000 | 30 | - |
| 78 | TSS | +++ | - | - | - | - | +++ | 1200 | 18 | - |
| 81 | TSS | +++ | - | - | - | - | - | 2090 | 11 | - |
| 87 | TSS | +++ | + | - | - | - | ++ | 8500 | 14 | - |
| 90 | TSS | +++ | + | - | - | - | +++ | 6250 | 9 | - |
| 91 | TSS | +++ | + | + | - | - | + | 2900 | 20 | - |
| 93 | TSS | + | + | + | - | - | +++ | 520 | 11 | - |
| 99 | TSS | + | + | - | - | - | + | 1160 | 6 | - |
| 100 | CLL | - | - | - | - | - | - | 240 | 15 | - |
| 79 | GLH | ++ | - | + | + | + | +++ | 280 | 36 | <i>S. mansoni</i> |
| 95 | NDD | - | - | - | - | + | - | 250 | 13 | - |
| 92 | NDD | - | - | - | - | + | +++ | 60 | 23 | - |
| 69 | NDD | - | - | - | - | + | +++ | 136 | 23 | ? Tuberculosis |
| 74 | NDD | - | - | - | - | + | +++ | 60 | 12 | - |
| 88 | NDD | - | - | - | - | - | ++ | 230 | 29 | - |
| 96 | MD | + | ++ | + | - | - | - | 900 | 10 | Portal hypertension |
| 86 | MD | - | - | - | - | - | - | 200 | 13 | Tuberculosis |

Table XI. Details of Four Patients with gross lymphoid hyperplasia (GLH – not TSS or CLL)

| Series No. | Age | Sex | Tribe | Spleen cm | Hb. g/100 ml | Lyc. count | Lyc. response <i>in vitro</i> | HSL | PI | IgM IU/ml | IgG IU/ml | IgA IU/ml | C3 | Notes | |
|------------|---------------|-----|-------|-----------|--------------|------------|-------------------------------|-------|-----|-----------|-----------|-----------|----|-------|------------------------------------|
| | | | | | | | | | | | | | | | % blast |
| 6 | pre-treatment | 25 | F | H | 24 | 5.4 | 130,000 | 0→90 | nd | nd | 25 | 205 | 45 | 82 | <i>P. falciparum</i> |
| | | | | | | | | | | | | | | | |
| 28 | pre-treatment | 30 | F | H | 20 | 6.0 | 165,000 | 5→66 | + | +++ | 142 | 284 | 50 | 130 | Marrow: "CLL" |
| | | | | | | | | | | | | | | | |
| 41 | pre-treatment | 51 | F | H | 28 | 7.0 | 1,000 | 13→63 | +++ | +++ | 220 | 450 | 80 | 80 | Marrow: pleomorphic lycs |
| | | | | | | | | | | | | | | | |
| 79 | pre-treatment | 40 | F | H | 36 | 9.3 | 68,000 | 18→35 | +++ | +++ | 280 | 189 | 35 | 74 | Lymph node: lymphoblastic lymphoma |
| | | | | | | | | | | | | | | | |

Lyc. = lymphocyte; C = control culture, not stimulated by PHA; P = culture stimulated by PHA; HSL = hepatic sinusoidal lymphocytosis; PI = portal infiltration with lymphocytes; treatment = proguanil 100 mg daily; nd = not done; H = Hausa.

Table XII. Details of Thirteen Patients with no Definite Diagnosis (NDD)

| Series No. | Spleen cm | IgM IU/ml | PHA % blasts | HSL | PI | Other findings and comments | Possible diagnosis |
|------------|-----------|-----------|--------------|-----|-----|--|-----------------------------------|
| 44 | 11 | 728 | 41* | –* | + | Lyc. 230, xs lyc. in marrow | Lymphoma |
| 45 | 8 | 728 | 6* | –* | +++ | Hb. AS. Chicken pox. | Lymphoma |
| 95 | 13 | 250 | 88 | –* | + | | ? |
| 4 | 8 | 80* | 4* | ++ | | <i>P. falciparum</i> *, Prog+ | Malaria in immunosuppressed adult |
| 25 | 22 | 169 | 79 | + | + | Prog+, took proguanil for 3 months. Spleen Ocm 3 years later. | Early TSS |
| 40 | 7 | 284 | 86 | + | – | No change after 20 months without treatment*, xs lyc. in marrow. | |
| 52 | 31 | 160* | 93 | + | +++ | FF, Prog–*, Hb AS, 49% spontaneous blasts in culture, xs lyc. in marrow. | Lymphoma |
| 57 | 12 | 220 | 70 | + | +++ | Prog–* | ? |
| 69 | 23 | 136* | 98 | +++ | +++ | Lyc. 18 000/ul, 87% spontaneous blast in culture, xs lyc in marrow, hepatic granulomas* | Tuberculosis (absconded) |
| 74 | 12 | 60* | 92 | +++ | ++ | FF, high fever*, xs lyc in marrow. | Lymphoma |
| 88 | 29 | 230 | 94 | ++ | +++ | Haemozoin ++, Eosinophilia, high fever*. | Schistosomiasis |
| 92 | 23 | 60* | 98 | +++ | +++ | Nodes ++, normal histology, IgG 87 IU/ml, IgA 18 IU/ml, Hb. 6.6 g*/100 ml. Died, no autopsy. | Lymphoma |
| 98 | 20 | 90* | 95 | + | +++ | Megaloblastic anaemia. | Lymphoma |

*=Feature against diagnosis of TSS. HSL=Hepatic sinusoidal lymphocytosis. PI=Portal infiltration. Prog+/-=Positive/negative response to proguanil. Lyc=Lymphocytes, total peripheral or excess (xs). FF= Fulani female.

Compared with the TSS group, this group had a normal sex ratio and age range, a low Fulani ratio (2/13), equivalent spleen sizes, 5 low IgM values and 3 poor responders to PHA. Malaria parasites (*P. falciparum*) were present in 2/10 of 2/31 (*P. malariae*) in TSS. The livers showed no unusual preponderance of PI and greater number with malarial pigment (Table VII). Ten cases had HSL, but each of these had at least one feature denying a diagnosis of TSS. One patient only might have been a possible early case of TSS.

Discussion

This study has shown four things: first that TSS is common in northern Nigeria, accounting for 40% of patients referred on account

of splenomegaly (Table I); secondly, there is a group of patients with gross lymphoid hyperplasia (GLH) who do not fit into any of the usual categories; thirdly, that 17% of patients remain undiagnosed even after extensive investigation; and fourthly, the study has helped further to define TSS and its pathogenesis and that of other splenomegalies in northern Nigeria.

Diagnosis and management of tropical splenomegaly syndrome

A female Fulani patient with splenomegaly has a 75% chance of having TSS. This approaches 100% if her IgM is over 760 IU per ml, the local upper limit of normal. The Fulani preponderance may reflect a genetic susceptibility or may be a consequence of a nomadic life in the northern Savannah. Hausas and other tribes, by contrast, have a 25% chance of having either TSS or leukaemia or lymphoma when presenting with splenomegaly. All four patients designated GLH were Hausa females.

There was a suggestion of a bimodal age distribution of TSS, with one group of nine patients below twenty years and the majority clustered around the fourth and fifth decades (Table II). The two sexes were about equally represented under the age of twenty, but females predominated at other ages. It may be that TSS incidence reflects (1) endocrinological changes during puberty and (2) alterations in immunity in older women, possibly the late effects of reduced resistance to malaria during pregnancy (GILLES, LAWSON, SIBELAS, VOLLER & ALLAN, 1969). A comparison of parity of women with TSS and women without enlarged spleens in the same community could be of interest. Sick cell disease or acute leukaemia were more likely causes of gross splenomegaly under the age of twelve.

TSS was found in patients with only moderate degrees of splenomegaly (Table III), and neither the size of the spleen and liver nor the presence of lymphadenopathy was of diagnostic value.

Lymphocytosis in blood, bone marrow or liver has to be interpreted most cautiously (Table IV–VII). The presence of frankly malignant cells or smudge cells may be diagnostic of lymphosarcoma, but pleomorphic lymphocytes, at times almost indistinguishable from lymphoblasts, were a common finding in TSS, GLH, NDD and miliary tuberculosis, but not in CLL. The close correlation between pleomorphism in smears and high blast counts in cultures unstimulated with PHA suggested very active lymphocyte stimulation by an agent, either antigenic or mitogenic.

The blood counts of rural symptom-free Africans (including the Zaria blood donors) differ in several ways from normal values estab-

lished in industrial countries (Table IV). The Hb is on average 2 grams per 100 ml lower for both sexes and all ages (GILLES, 1964). There is a neutropenia (EZEILO, 1971), eosinophilia is common and there is a thrombocytopenia. The average spleen weight in Lagos is twice that in London (BRUCE-CHWATT, 1956), and a degree of hypersplenism may contribute to the relative anaemia, neutropenia and thrombocytopenia, although there is also some evidence of a racial neutropenia in Negroes (AKINYANJU & GROSSMAN, 1973).

Peripheral blood counts in patients with TSS, deviated from normal in three ways:

1. the lymphocytosis (Table V) was of primary importance, being directly to the supposed aetiology of the condition;
2. there was an exaggeration of the anaemia, neutropenia and thrombocytopenia found in the symptom-free (Table IV) and this was attributed to a secondary effect of hypersplenism;
3. there were coincidental conditions, including the eosinophilia of parasitic infections, iron deficiency and folate deficiency (Table VI).

Iron deficiency is known to be common amongst Fulani, even in the absence of hookworm infection (BELL & HOWELLS, 1973), and they predominated amongst the TSS patients. Increased requirements for folate due to erythroid hyperplasia secondary to haemolysis may have contributed to the one third prevalence of megaloblastosis.

Liver biopsy was not diagnostic in TSS in our patients (Table VII) nor those of SAGOE (1970), but the condition should be suspected if there is hepatic sinusoidal lymphocytosis, hypertrophied Kupffer cells some of which look rather "ragged", an absence of malarial pigment, and a mild lymphocyte and monocytic infiltration of the portal tracts. Hepatic sinusoidal lymphocytosis has also been described in Europeans who have never visited the tropics (DACIE, BRAIN, LEWIS & WORLLEDGE, 1969; BLENDIS, ANSELL, JONES, HAMILTON & WILLIAMS, 1970) and could be a response secondary to severe reticuloendothelial barrage of any origin (ZIEGLER, 1973). The four typical TSS patients without HSL may have been early cases, but their spleen size and IgM values did not support this suggestion. Liver biopsies of patients with TSS usually showed mild or moderate lymphocytosis in the portal tracts, but gross lymphocytosis was more suggestive of other diagnoses; this was in agreement with the Ibadan findings (SAGOE, 1971). The haemozoin pigment seen so commonly in liver biopsies in northern Nigeria was probably of malarial origin (EDINGTON, in preparation), and may reflect the relative instability of malaria in the north. The relative absence of malarial pigment in livers of TSS was further evidence of hyperimmunity to malaria in these patients (Table VII).

The small amount of iron pigment noted in liver biopsies was surprising as a degree of haemolysis was usually present in TSS. It is

probable that the haemosiderin released by the break down of the erythrocytes was re-utilized rapidly as many of the patients were iron deficient (Table VI). It has been noted already that iron pigment is unusual in the livers of children suffering from severe malarial infections and a similar mechanism has been postulated.

Immunoglobulin results proved the single most useful diagnostic investigation (Table VIII; Figure 2). IgM levels above 760 IU per ml were extremely suggestive of TSS while values below 343 IU per ml (the normal mean) virtually excluded the diagnosis. It is necessary to have an adequate, carefully selected, matched, normal population for the provision of normal values if results in disease are to be interpreted. In our immediate neighbourhood Zaria city dwellers, Hausa farmers and Fulani cattle herders had significantly different values of IgM and IgG (MOHAMMED, TOMKINS & GREENWOOD, 1973; this study), and we have pooled the rural values to give as close a fit as possible with the majority our patients. High IgM values in TSS were in agreement with all other series (CHARMOT & ANDRÉ, 1964; WELLS, 1970; SAGOE, 1970; ZIEGLER, 1973). High IgM values were found in other patients and we did not find the clean cut off that SAGOE (1970) did between TSS and the rest. Abnormally low IgM or IgG values were found helpful in suggesting or confirming a diagnosis of heukaemia or lymphoma (FIDDES, PENNY, WELLS & ROZENBERG, 1972). Low IgM excluded TSS even in the face of hepatic sinusoidal lymphocytosis and response to proguanil. Low IgA levels, though common in these disorders, were difficult to interpret because they were low also in TSS, a feature which has been reported from New Guinea (WELLS, 1970). Serum complement levels were depressed only in association with very high IgM values (Table IX; Figure 3).

ZIEGLER (1973) showed a correlation between IgM in Kupffer cells, serum IgM levels and spleen size in Ugandan patients with TSS. The presence of IgM detected by immunofluorescence in Kupffer cells of snap-frozen liver biopsies proved in our patients to be virtually diagnostic of TSS (Table X). Unfortunately this test requires more sophisticated equipment and techniques than are available in many tropical hospitals.

Lymphocyte response to PHA has been of limited diagnostic help because, unlike SAGOE (1970), we did not find that patients with leukaemia or lymphoma necessarily showed impaired blastic transformation, although a response of less than 40 per cent was clearly abnormal and excluded TSS (Figure 1). Interestingly and by contrast, thymidine uptake by stimulated CLL lymphocytes was consistently low. The relative merits of these two methods of investigating lymphocyte responsiveness have been reviewed by VALENTINE (1971). In our hands, blast counting was the more useful test.

A clinical response to proguanil, though consistent in TSS was not confined to it (Figure 4). This result differed from SAGOE's (1970) and there are several possible explanations. The response to proguanil in some patients without TSS could have been a reflection of a relative instability of malaria in the north compared to the south of Nigeria. The patients with malignancies of the lymphoreticulo-endothelial system may have had a lowered resistance to malaria, resulting in a lymphoid hyperplasia other than that directly the result of their malignancy and similar to that seen in the non-immune; this secondary lymphoid hyperplasia would account for the greater spleen size in African patients with CLL than with CML (OMO-ADUA & FLEMING, 1974) which reduces with proguanil alone without cytotoxic therapy (ALLAN & WATSON-WILLIAMS, 1963). A non-specific immunosuppressive action of proguanil has been postulated (SAGOE, 1970; STUIVER, ZIEGLER, WOOD, MORROW & HUTT, 1971), but has never been demonstrated.

Additional features against a diagnosis of TSS included oedema, jaundice, bleeding, high fever, severe anaemia (Hb < 6.7 g per 100 ml), *P. falciparum* infection and objective response to paludrine unaccompanied by subjective improvement.

There has been no place for splenectomy in patients with proven TSS in this series.

The pathogenesis of TSS

The essential features of TSS in northern Nigeria are:

1. splenomegaly in the presence of acquired immunity to malaria;
2. raised serum IgM;
3. the presence of IgM fixed in Kupffer cells;
4. lymphoid hyperplasia, to be seen in the peripheral blood, bone marrow and hepatic sinusoids;
5. full clinical and haematological response to proguanil.

Pathogenesis should be studied only in patients who fulfill these criteria, and who have no other condition likely to complicate the picture.

There seem to be certain differences in the patterns of TSS between different geographical areas. In the Watut valley of New Guinea, TSS is exceptionally common, having been reported in about 20 per cent of the adult population (PRYOR, 1967a) and 27 per cent of the patients do not respond to antimalarials (CRANE, HUDSON & HUDSON, 1973). In Uganda, there are definite tribal (HAMILTON, HUTT, WILKS, OLWENY, NDAWULA & MWANJE, 1965) and familial associations (ZIEGLER & STUIVER, 1972) and less obvious lymphoid hyperplasia (MARSDEN, HUTT, WILKS, VOLLER, BLACKMAN, SHAH, CONNOR, HAMILTON, BANWELL & LUNN, 1965). However, it seems likely that several other series (WATSON-WILLIAMS & ALLAN, 1968; PRYOR, 1967; LOWENTHAL,

O'RIORDAN & HUTT, 1971; STUIVER, ZIEGLER, WOOD, MORROW & HUTT, 1971; CRANE, HUDSON & HUDSON, 1973) included patients we would have excluded using our much stricter criteria and diagnostic methods previously not available.

It is not known whether the raised IgM level or the lymphoid hyperplasia occurs first in developing TSS. IgM levels did not correlate with spleen size in our patients or those of SAGOE (1970), especially when splenomegaly was gross. The reduction in IgM levels following proguanil therapy suggested that IgM was overproduced rather than slowly metabolised. The presence of macroglobulins in TSS has been known for a long time: they are essentially cryoglobulins containing mainly IgM and sometimes smaller quantities of IgG, IgA and complement, presumably complexed (CHARMOT & VARGUES, 1963; WELLS, 1970; ZIEGLER, 1973). We have confirmed that these IgM macroglobulins can be detected in fixed reticulo-endothelial cells, but it is not known whether this is a physiological sequel to high circulating IgM levels or whether it is a pathological feature of TSS. We have been unable to identify "early" cases of TSS. ZIEGLER and STUIVER (1972) found raised IgM levels in their TSS "kindred group", and ZIEGLER (1973) later showed a clear correlation between raised IgM, splenomegaly, cryoglobulinaemia and IgM phagocytosed by Kupffer cells in patients with TSS. Which of these abnormalities came first, however, was not established.

Between 1–2 per cent of a population about 150 miles (240 km) north east of Zaria have IgM values in excess of 2,000 IU per ml. but do not have palpable spleens, and their IgM levels fall with anti-malarial treatment (CORNILLE, BROGGER & MATSUSHIMA, 1974). The examination of liver biopsies in such people for the presence of phagocytosed IgM, and long term follow up might solve this problem. It would be necessary also to examine their serum for the presence of auto-antibodies to heart muscle and gastric parietal cells antibodies, which are not found in TSS (WELLS, 1970) but are seen in a different non-splenomegalic macroglobulinaemia syndrome in Uganda (SHAPER, KAPLAN, MODY & MCINTYRE, 1968).

The cause of the increased IgM production is not understood (WELLS, 1970). The idea that there might be a genetically determined predisposition to produce predominantly IgM antibody with an inability to switch to IgG production (ZIEGLER & STUIVER, 1972) did not receive support from CRANE, ROWLEY, WARBURTON and MACKAY (1972) who showed that the IgG antibody response, which is the secondary antibody response, to flagellin was not preferentially impaired in patients with TSS in New Guinea.

Our results add support to ZIEGLER's (1973) contention that TSS represents an immune complex disease whereby IgM is complexed by

anti-IgM and phagocytosed. This provides a reticuloendothelial barrage which is followed by lymphocytosis and splenomegaly. Hepatic sinusoidal lymphocytosis may represent a subsequent immune response to phagocytosed complexes or some component thereof (STUIVER, ZIEGLER, WOOD, MORROW & HUTT, 1971). Electron microscopic studies of hepatic sinusoids in TSS support this view (FLUCK, HUTT, FLUCK & STUIVER, 1973).

The evidence of a relationship between malaria and TSS (PITNEY, 1968; *Brit. med. J.*, 1969) although not convincing is fourfold: epidemiological, the presence of high titres of antimalarial antibodies, the response to antimalarials and an under-representation of Hb AS. It would be tempting to explain differences in the pattern of TSS in different places by the different patterns of malarial transmission and endemicity, and in this study we have presented further evidence of increased immunity to malaria in patients with TSS. If there is such a relationship, how does malaria trigger off TSS?

One suggestion is that TSS represents a state of hypersensitivity to sequestered malarial antigen, accompanying a more-than-usually efficient immunity to malaria. Light and electronmicroscopic histology of liver biopsies suggest that hypersensitivity would be cellular and would presumably therefore be a response to sequestered malarial antigen in Kupffer cells. In this situation, the gross overproduction of IgM antibodies, which include antibodies to sheep erythrocytes, altered human gammaglobulin (WELLS, 1970) and malaria (ZIEGLER, VOLLER & PONNUDURAI, 1973), is an adjuvant effect. Other diseases where such an adjuvant effect of reticuloendothelial barrage is seen include lepromatous leprosy, visceral leishmaniasis (TURK & BRYCESON, 1971) and classical chronic malaria (TURNER & VOLLER, 1966). However, the exaggerated antibody production is of the class IgG in these diseases. The overproduction of IgM in TSS resembles that seen in trypanosomiasis, and GREENWOOD (1974) has suggested that in that disease, the stimulus may be coming not from a specific antigen, but from a mitogen which non-specifically stimulates antibody production by B lymphocytes (ROITT, GREAVES, TORRIGIANI, BROSTOFF & PLAYFAIR, 1969).

Such an hypothesis could also explain the apparent absence of anti-malarial antibody in cryoglobulins in TSS (ZIEGLER, 1973). We are currently investigating the antigenic and mitogenic effects of malarial parasite extracts in patients with TSS.

Diagnosis of chronic lymphatic leukaemia and lymphoma

The diagnosis of CLL may be suggested by classical blood and bone marrow appearances, but can only be confirmed in areas where TSS is

found when there is evidence of gross immunological impairment, as shown by lowered Ig levels or reduced lymphocyte responsiveness to PHA. In this we confirm SAGOE's (1970) observations and have found no evidence to suggest that TSS is ever a preleukaemic condition.

About 10 per cent of patients with TSS in West Africa have blood and marrow appearance indistinguishable from CLL. These patients could explain the reported high female incidence, and absence of lymphadenopathy in Africans diagnosed as CLL by some (ALLAN & WATSON-WILLIAMS, 1963; EDINGTON & MACLEAN, 1964; HADDOCK, 1967), though not by others (LOTHE, 1967; KASILI, CARDWELL & TAYLOR, 1969).

We have postulated above that in CLL, lymphoma and other conditions of impaired resistance to malaria, the clinical and haematological manifestations of malaria will respond to antimalarials. The suggestion that TSS is pre-malignant (WATSON-WILLIAMS & ALLAN, 1968) could arise when the lymphoid hyperplasia responds to proguanil before the underlying lymphoma reveals itself in other ways. Partial response or failure of response of TSS to proguanil in New Guinea (CRANE, HUDSON & HUDSON, 1973) and elsewhere may also have been due to other complicating factors or underlying disease. All proven cases of TSS in Africa respond to antimalarials (SAGOE, 1970; STUIVER, ZIEGLER, WOOD, MORROW & HUTT, 1971; this series).

Patients with gross lymphoid hyperplasia

The four cases called simply GLH fell into a group because they presented a common problem. They had features which both supported and denied the diagnoses of TSS and CLL at the same time (Table XI). Their essential common features are:

1. multiparous Hausa females – like CLL, unlike TSS;
2. huge spleens – like TSS and CLL;
3. very high lymphocyte counts in two cases – like CLL and not typical of TSS;
4. intense lymphocytosis of bone marrow, hepatic sinusoids and portal tracts – more like CLL than TSS;
5. very active lymphocytes as judged by blood and bone marrow smears and *in vitro* blast transformation in unstimulated cultures – like TSS, unlike CLL;
6. normal lymphocyte response to PHA, assessed both by blast transformation and by 3H-thymidine incorporation – like TSS, unlike CLL;
7. normal immunoglobulins, except for one low IgM value – unlike TSS, unlike CLL;

8. normal serum complement levels – like CLL, unlike TSS;
9. clinical and haematological response to proguanil in the three patients who were followed up for periods of 7, 11, and 16 months: the response consisted of a steady improvement in well-being, spleen size and haemoglobin concentration, and a steady fall in lymphocyte counts in two patients – like TSS, unlike CLL.

The four patients also had certain dissimilar features: one had heavy *P. falciparum* infection and low IgM concentration, one had no lymphocytosis in the blood and was lost to follow up, and one had a lymph node biopsy suggestive of lymphoblastic lymphoma. The latter patient's picture was complicated by a short course of cyclophosphamide (100 mg daily for eight weeks) given at a time when she was already showing rapid improvement on antimalarials alone. She has been treated now with proguanil alone for one year and improvement has continued. This patient also had IgM, IgA and complement fixed in Kupffer cells. Despite these differences we suggest that these patients be considered as a group.

There is no good evidence that these patients have ever had TSS, although in one instance (no. 79, table XI) IgM, IgA and complement were detected in Kupffer cells. We suggest that they are suffering from a lymphoid hyperplasia of a quite different origin, which is associated with impaired immunity to malaria and may perhaps proceed to leukaemia or lymphoma. A possible sequence of events would be that during pregnancy, immunity is lowered to malaria (GILLES, LAWSON, SIBELAS, VOLLER & ALLAN, 1969) and also to a potentially leukaemogenic agent, perhaps a virus. SCHOFIELD, PARKINSON & KELLY (1964) have shown that women of child bearing age are especially susceptible to malarial anaemia and splenomegaly, and that splenomegaly tends to persist even after malaria has been controlled by DDT spraying. Malaria further depresses the immune response (GREENWOOD, BRADLEY-MOORE, PALLIT & BRYCESON, 1972) and lowers resistance to the leukaemogenic agent (WEDDERBURN, 1970). The infections initiate the lymphocytosis. At this stage, the lymphocytes are not malignant. If immunological function remains adequate natural immunity may still overcome the infection. If immunological function becomes severely depressed the condition progresses and permits further attacks of malaria. A vicious cycle ensues and the leukaemogenic potential of the virus is expressed. Treatment with proguanil breaks this cycle and natural immunity again has a chance to overcome the infection. A similar association between malaria and Burkitt's tumour has already been suggested on epidemiological grounds (KAFUKO & BURKITT, 1970).

Patients with no definite diagnosis

It is to the group in whom no definite diagnosis (NDD) could be made that one must look for patients with early TSS. Careful analysis allows only one possible patient (no. 25, Table XII), whose liver biopsy was not examined for the presence of phagocytosed IgM. The other patients are a heterogeneous collection. Six of them have evidence of immunological deficiency (low Ig values or impaired response to PHA), and on SAGOE's (1970) experience they might be expected to have lymphoma. Alternatively they could have undiagnosed portal hypertension due to portal vein thrombosis, a condition common in Kenya (BAGSHAWE, 1970) and probably under-represented here, or they may represent the normal upper limit of splenomegaly in a relatively unstable malarial area. The size of this group, 31 per cent of all our patients, is disappointingly high. Possibly earlier recourse to laparotomy would have produced more definite diagnoses.

Conclusions

There seem to us to be three categories of patient in northern Nigeria who have marked lymphoid hyperplasia. There is one group (TSS) with a non-leukaemic response to a B cell mitogen or a true hypersensitivity to malarial antigen sequestered in reticuloendothelial cells. There are malignant lymphomas, including CLL, which may be accompanied by a second lymphocytosis of malarial aetiology. There is a group we call GLH there in which there is a postulated association between a state of immunosuppression, malaria and a possible leukaemogenic agent which does not necessarily proceed to leukaemia. All three groups may respond or seem to respond to proguanil.

The accurate diagnosis of these conditions can be difficult. TSS is easily mistaken for CLL or lymphoma and lymphoma for TSS; lymphocytosis due to malaria overlies that due to lymphoma and masks the true diagnosis; the lymphocytosis of GLH may or may not be pre-malignant.

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