Figure 4

Figure 5

FIG. 4.—Total gastric resection for “doubtful” high lesser curve ulcer. FIG. 5.—Sites of gastric resection in “doubtful” mid-lesser curve ulcer.

gastric resection (the Kelling-Madlener operation) or preferably by vagotomy and simple drainage.

When the “doubtful” ulcer is on the mid-lesser curve the surgeon, not wanting to perform a very high gastric resection, may choose to divide the stomach a little distance above the ulcer (Fig. 5). If in this case the ulcer proves to be malignant then the operation was inadequate. If it proves to be benign then the gastric resection again was unnecessary. If in this same case the surgeon decides on a high gastric resection

because he has doubt about the nature of the ulcer then again a serious error has been made, for the patient is left with very little stomach and all the disabilities and sequelae which may follow such a high resection.

How, then, is the problem to be solved? There would seem to be three ways of dealing with it. The first is to treat the patient on a medical regimen for a matter of weeks and to follow the course of healing. All benign gastric ulcers, especially large ones, are best allowed to heal before selective vagotomy is undertaken. Selective vagotomy is easier when the ulcer is completely healed. The second method is to excise the ulcer completely at the time of vagotomy and to use frozen section or await the “paraffin” report. The third method is to open the stomach and palpate the ulcer edge with the finger, and to take one or more biopsy specimens from the edge, using frozen section, or, failing this, to perform the lesser operation of vagotomy and simple drainage, then await the paraffin report. This is the method advised by Dragstedt and the one which we have chosen, so far without regret. The more carefully this method of biopsy is practised the fewer mistakes will arise. In fact there should be none. As with most mistakes in surgery the fault lies not with the method but with how well it is carried out. Probably, very rarely a mistake will arise and a malignant ulcer will be overlooked. The late results of gastric cancer are not good, and the harm done by a very occasional error must be weighed against that arising from routine gastric resection sometimes radically and unnecessarily done for benign ulcer.

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Tropical Splenomegaly Syndrome: Long-term Proguanil Therapy Correlated with Spleen Size, Serum IgM, and Lymphocyte Transformation*

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Summary: Forty-three patients with an initial diagnosis of tropical splenomegaly syndrome were placed on long-term proguanil therapy. All patients who failed to respond to proguanil and who were adequately followed up developed identifiable disease, usually malignant lymphoma or chronic lymphatic leukaemia. In patients who responded to proguanil IgM values were always very high and phytohaemagglutinin (P.H.A.)-lymphocyte-transformation scores were always normal before treatment was started. In patients who failed to respond IgM values were within the normal range or below, while P.H.A.-lymphocyte-transformation scores were abnormally low. During proguanil treatment IgM values fell gradually, closely paralleling the decrease in spleen size.

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Introduction

A syndrome of chronic splenomegaly without definable aetiology is found in some people living in tropical areas and has been reported from the Congo (Charmot and Vargues, 1963), Uganda (Marsden *et al.*, 1965), Sudan (Mustafa, 1965), Zambia (Lowenthal *et al.*, 1966), New Guinea (Pryor, 1967) and Nigeria (Edington, 1967; Watson-Williams and Allan, 1968) and reviewed by Pitney (1968). Increased values for serum IgM in this syndrome have been reported from the Congo, Algeria, and the Ivory Coast (Charmot and André, 1964) and reviewed by (Trincão *et al.*, 1966) and from New Guinea (Wells, 1968). Hepatic sinusoidal lymphocytosis was found in many of the liver biopsy specimens from Uganda and New Guinea (Marsden *et al.*, 1965, 1967). In Ibadan it has been found that the lymphocytosis is not limited to the liver but also involves the peripheral blood and bone marrow

of patients with this syndrome. In a previous paper from this department Watson-Williams and Allan (1968) reported that a large majority of patients responded with complete remission to the prolonged administration of the antimalarial drug proguanil (Paludrine).

The diagnosis of the syndrome is by exclusion of all other causes of splenomegaly encountered in tropical areas. Moreover, the differential diagnosis from a malignant lymphoma, and in Ibadan chronic lymphatic leukaemia in particular, can be a difficult problem when there is no palpable lymphadenopathy. This study is designed to give, if possible, positive criteria for the diagnosis of the syndrome. Clinical and biochemical findings of patients with an initial diagnosis of tropical splenomegaly syndrome (T.S.S.) are given. Before starting antimalarial therapy, observations were made of the response of each patient's peripheral blood lymphocytes to stimulation by phytohaemagglutinin (P.H.A.) and their serum IgM levels, and during therapy serial estimations of serum immunoglobulin levels were made. The fate of those who failed to respond to antimalarial therapy is recorded.

Patients and Methods

Adult patients were admitted to this study only if they fulfilled the following criteria:

(a) Splenomegaly of more than 10 cm. measured from the costal margin (at the anterior axillary line) to the apex of the spleen. This corresponds to grade 4 or more of the Hackett classification (World Health Organization, 1963).

(b) Exclusion of other causes of gross splenomegaly as follows: (i) Bacterial infections by examination of blood and stool; x-ray, culture of sputum and in some cases culture of bone marrow and splenic aspirates for *Mycobacterium tuberculosis*. (ii) Parasite infections by repeated examination of thick films for malaria parasites; stool and urine examination for ova of schistosomes; marrow culture and in a few cases splenic cultures for *Leishmania*. (iii) Primary blood dyscrasia by full haematological examination of blood and bone marrow. (iv) Liver function tests, liver biopsy (32 cases), and splenoportograms (12 cases) were done to exclude primary liver diseases. Hepatic sinusoidal lymphocytosis (H.S.L.) in liver biopsies was graded according to Marsden *et al.* (1967).

Determination of Immunoglobulins

Commercially prepared immunoplates (Hyland Laboratories, California, U.S.A.) were used for the estimation of IgG, IgA, and IgM according to the method of Fahey and McKelvey (1965). Three standard sera of known concentration (supplied by Hyland) were included in each plate. Whenever the precipitin rings obtained with IgM tests were larger than those obtained with the standards the original sera were diluted 5-20 times with phosphate-buffered saline pH 7.2, so that the ring diameters would fall within the range found with the standards used.

In order to confirm the high values of IgM in many of the sera, the same method was employed but with specially prepared immunoplates, using lyophilized anti-IgM† so that the concentration of antibody incorporated per ml. of agar was increased threefold.

P.H.A. Transformation

Phytohaemagglutinin (P.H.A.) "Wellcome" was used to initiate blastic transformation of peripheral blood lymphocytes in culture with the method suggested by Wellcome Research Laboratories, Beckenham, with modifications. The cells were harvested after 72 hours and slide preparations were stained

with May-Grunwald-Giemsa. From each culture 500 cells were counted, being scored according to the method suggested by Pentecross (1968).

Results

Forty-three patients fulfilled initially the criteria for the diagnosis of tropical splenomegaly syndrome‡ (as set out under Methods). All patients were treated with proguanil, 100 mg./day, for at least six months. At the end of this period a marked regression in spleen size was recorded in 32 patients, hereinafter referred to as "responders." No reduction, but sometimes an increase, in spleen size was noted in the remaining 11 patients, hereinafter referred to as "non-responders."

Clinical and Haematological Findings.—All patients complained of abdominal swelling with discomfort of a few months to several years' duration. Mild symptoms of anaemia were present in the majority. The anaemia was normocytic and normochromic. The total white blood cell count varied widely, but the typical feature, especially in responders, was a lymphocytosis in the peripheral blood and bone marrow which also showed normoblastic hyperplasia. The platelet count was often below normal, but there was no evidence of bleeding diatheses. Repeated thick blood films for malaria parasites were negative.

Immunoglobulin Values (Table I).—IgG and IgA: No significant difference was found in the levels of IgG and IgA in

TABLE I.—Serum Levels of Immunoglobulins in 43 Patients with an Initial Diagnosis of T.S.S., Shown in Those Who Responded and Those Who Did Not Respond to Proguanil Therapy. Spleen Sizes Range from 10-30 cm. in Both Groups. In Responders the Spleen Sizes During Therapy are Expressed as a Percentage of the Initial Value

Group	Spleen Size as a Percentage of Initial Measured Value	IgG (mg./100 ml.)		IgA (mg./100 ml.)		IgM (mg./100 ml.)	
		Range	Mean	Range	Mean	Range	Mean
Responders (32)	100	750-5,100	2,175	150-300	185	860-3,000	1,799
	50	1,050-4,400	2,443	78-210	130	310-860	553
	0	1,050-4,000	1,972	45-260	152	20-420	308
Non-responders (11)	100	750-3,800	1,810	48-400	135	20-325	81
Normal adult Nigerians*		1,250-4,100	2,500	60-360	183	50-400	189

*40 blood donors without splenomegaly.

our patients and the controls. IgM: Before treatment the responders had 3-10 times the values for normal adult Nigerians, while all the non-responders had serum IgM levels either within or below the normal range. A scattergram of the initial IgM values against the initial values of the spleen size is shown in Fig. 1. It can be seen that the Individual IgM values bear no correlation to the size of the spleen in either group, and that responders have much higher IgM values than the non-responders. The non-overlap of IgM values found in this study between the group of T.S.S. patients and the control group is in contrast to the findings of Wells, (1968) in New Guinea. This can be explained by the selective nature of the patients in this study: all had splenomegaly of grade 4 or more; all were investigated to exclude other causes of splenomegaly, and all made significant response to proguanil therapy.

Lymphocytes and P.H.A.-induced Lymphocyte Transformation.—Absolute lymphocyte counts varied widely, depending on the total white cell count (W.B.C.). Values of 1,380-35,000

† Supplied by Professor Houba of the W.H.O. Immunology and Research Training Centre, Ibadan.

‡ None of these was included in the series published by Watson-Williams and Allan (1968).

FIG. 1.—Initial spleen sizes and corresponding serum IgM values.

FIG. 2.—Hepatic sinusoidal lymphocytosis. Percentages refer for the cases of splenomegaly to the total number of 32 (responders plus non-responders); for the “controls” to the total number of 24 post-mortem biopsies from cardiac and neurological cases without splenomegaly.

cells/cu.mm. in responders and 1,150-56,000 in non-responders were recorded. The biological behaviour of these lymphocytes in culture with P.H.A. was tested in 35 patients (Table III); 25 of the 32 responders tested, including 5 cases with W.B.C. of over 20,000, had normal scores, and 10 of the 11 non-responders tested had abnormally low scores.

Liver Biopsies were performed on 32 patients (25 responders to antimalarials and 7 non-responders). Of the responders 21 had hepatic sinusoidal lymphocytosis (H.S.L.) of various grades (unrelated to their spleen sizes) while four had normal biopsies. Three of the non-responders had H.S.L. and four had normal biopsies. Lymphocytic infiltration of portal tracts was observed in five of the non-responders (three with and two without H.S.L.). One-third of the control subjects had H.S.L. (Fig. 2).

Effect of Proguanil Treatment.—Proguanil therapy caused a progressive diminution in spleen size, with haematological remission of initial anaemia, in 32 of the 43 cases admitted to this study. In responders it took from a few months to over two years for the spleen to reach the costal margin (that is, 0% of the initially measured size) (Fig. 3). The time

FIG. 3.—Effect of proguanil therapy on spleen size of patients with an initial diagnosis of tropical splenomegaly syndrome. Spleen size recorded as percentage of the initially measured size.

TABLE II.—Analysis of 11 Patients with Splenomegaly not Responding to Proguanil

Case No.	Sex	Initial Spleen Size (cm.)	IgM mg./100 ml.	P.H.A. Transformation Score in %	Effect of Proguanil on Spleen Size	Follow-up
1 ..	F.	24	50	4.6	None after 2 years. Increased terminally to 27 cm.	Terminal 2-week illness. Necropsy: Hodg-kin's sarcoma
2 ..	M.	18	325	5.0	No reduction after 18 months, then increased to 25 cm.	Developed haemorrhagic ascites; cytology showed lymphosarcoma cells. Lymphoma. Spleen reduced to 12 cm. in 2 months on chlorambucil
3 ..	M.	15	80	3.8	None after 18 months	Autoimmune haemolytic anaemia. Warm antibody type responsive to prednisolone. ? Lymphoma
4 ..	F.	15	80	Not done	None after 18 months	After 18 months developed autoimmune haemolytic anaemia responsive to prednisolone. ? Lymphoma
5 ..	F.	30	120	5.8	Increased to 39 cm. over 3 1/2 years	Terminal haemolytic anaemia and congestive cardiac failure. Defaulted and died at home. ? Lymphoma
6 ..	M.	27	20	1.0	None after 5 months	Rise in white blood cell count to over 100,000. Revised diagnosis of Chronic lymphatic leukaemia. Responding to chlorambucil
7 ..	F.	32	70	2.0	Initial reduction to 25 cm. in 1 month, then became static over 18 months.	As above: chronic lymphatic leukaemia
8 ..	M.	20	56	2.0	None after 4 months	As above: chronic lymphatic leukaemia
9 ..	M.	10	105	1.6	Increase to 14 cm. in 3 months	Defaulted and reported dead
10 ..	F.	30	20	2.6	None after 3 months	Defaulted and reported dead
11 ..	F.	14	64	6.0	None after 6 months	Defaulted

taken for the spleen to reach 50% of the initially measured size (the S_{50}) varied from 2 to 18 months. There is no straight-line correlation between the initial size of the spleen and the time to reach the costal margin. It is obvious, however, that the rate of decrease of the spleen is more closely related to the splenic mass than to the length of the spleen as measured here. In responders the serum IgM showed a progressive diminution as the spleen got smaller, while the values of IgG and IgA did not change significantly (Table I and Fig. 4). The rate of fall of IgM does not bear any simple relationship to the initial IgM value (Fig. 4). Subjects a, b,

FIG. 4.—Progressive serum IgM values while on proguanil therapy. The time required for fall of IgM to normal values is not proportional to initial values. For instance, Cases a, b, and c all had initial IgM values of 1,800 mg./100 ml.; cases x and y had initial IgM values of 2,600 and 1,600 mg./100 ml. respectively.

and c had identical initial values of 1,800 mg./100 ml., but the rate of fall of IgM for each was different: it took 4, 12, and 20 months respectively for each to reach normal levels. Similarly, x and y had IgM values of 2,600 and 1,600 mg./100 ml. respectively, but it took identical times (16 months each) to reach normal values. If, however, the serum IgM is plotted as a function of the spleen size (Fig. 5), there is a definite pattern in each individual—and in the group as a whole—

FIG. 5.—Progressive spleen sizes and corresponding IgM values while on proguanil therapy.

for the serum IgM values to reduce concomitantly with the reduction in spleen size (Table I). Fig. 6 summarizes some of the findings in a typical case of T.S.S. in response to proguanil. The diminution in spleen size was dramatic in

FIG. 6.—Response of a typical case of tropical splenomegaly syndrome to proguanil 100 mg./day.

the first few months and correlated closely with the decrease in IgM values. As the spleen size decreased, the anaemia was corrected and the peripheral blood lymphocytosis was reduced.

Fate of Non-responders.—This is summarized in Table II. All the 11 non-responders except Case 2 had low normal or below normal IgM values. Likewise, low lymphocyte transformation scores were obtained in the 10 that were tested. Of these 11 non-responders the diagnosis of T.S.S. was revised in eight, three having defaulted or died.

Discussion

In these 43 patients who all had a similar presentation, prolonged follow-up has produced a sharp separation into two groups—32 responding dramatically to treatment with proguanil, and 11 responding very little or not at all. By the end of four to six months every patient could be classified unambiguously as belonging to one or the other group. When these two groups were retrospectively analysed with respect to other features two major facts emerged. Firstly, responders had initially high IgM levels, thus confirming the findings of Charmot and Vargues (1963), Payet *et al.* (1963), and Wells (1968). These values were found to be much higher than those for non-responders. Secondly, responders had normal P.H.A.-induced lymphocyte transformation scores (in agreement with Ziegler *et al.*, 1969), whereas they were abnormally low in non-responders (Fig. 1 and Table III).

TABLE III.—Correlation Between Response to Proguanil, P.H.A. Transformation and IgM values

	IgM (mg./100 ml.)*	% P.H.A. Transformed Lymphocytes after 72 Hours†
Responders	860–3,000	64–88
Non-responders .. .	20–325	1–6
Normal adults .. .	50–400	60–80

*Determined in all 32 responders and 11 non-responders. †Determined in 25 responders, 10 non-responders, and 20 normal adult Nigerian blood donors.

Differential Diagnosis

These features seem of value, especially in relation to two differential diagnoses. Firstly, cases of malignant lymphoma

presenting with gross splenomegaly but without lymphadenopathy could be, and have been, misclassified as T.S.S. In such cases lack of a rise in IgM and abnormal P.H.A. transformation will militate against T.S.S. Secondly, cases of T.S.S. with a lymphocytic leukemoid reaction might be and have been misclassified as chronic lymphocytic leukaemia (Watson-Williams and Allan, 1968). In such cases high IgM and normal P.H.A. transformation will not favour such a diagnosis. Since these conditions differ widely in treatment of choice and in prognosis, it is important to differentiate between them as soon as possible—that is, before a time-consuming therapeutic trial.

The development of a neoplastic lymphoproliferative disorder could be established definitely in only five of the non-responders. Three others, however, had haemolytic anaemia of the type usually associated with malignant lymphomas; and three had a suggestive history and a fatal outcome (Table II). Nevertheless the possibility cannot be entirely ruled out that with continued reticuloendothelial hyperplasia in T.S.S. under certain host conditions or when timely therapy is not instituted, the cellular proliferation may proceed to a frank malignant proliferation.

Hepatic sinusoidal lymphocytosis (H.S.L.) found in most cases of T.S.S. was typical but not pathognomonic (Fig. 2). Some responders did not show this feature and a few normal controls had H.S.L., which could not be correlated with the degree of lymphocytosis in the peripheral blood as seen in Ibadan.

Proguanil Therapy in T.S.S.

Proguanil has been used for many years in T.S.S. in this hospital. It is safe and the mode of administration simple—one 100-mg. tablet daily. The response to proguanil consists not only in a decrease of spleen size (Fig. 3, and Watson-Williams and Allan, 1968), but also in a return to normal of haematological and IgM values (Figs. 4 and 6). Successful treatment with proguanil cannot be considered sufficient to incriminate malaria as the aetiological agent of the syndrome, for Houba and Adam (1964) have shown that the antimalarial drug chloroquine has additional—for example, antibacterial—actions. Whether proguanil has this combined effect is not certain, and neither is it known what effect, if any, proguanil may have in man on some proliferating cells or on cells actively producing immunoglobulins.

Source and Significance of Raised IgM

The spleen in T.S.S. constitutes the largest collection of lymphoreticular tissue in the body. In animals the spleen undergoes morphological changes characteristic of cells synthesizing proteins after antigenic stimulation (Hanna *et al.*, 1966); and van Furth *et al.* (1966) showed that human spleen cells can synthesize immunoglobulins in vitro. Notwithstanding the biosynthesis of IgM in other organs, the progressive diminution of serum levels of IgM accompanying the progressive diminution in splenic mass suggests that the abnormally large amounts of macroglobulins in T.S.S. may be formed in the spleen.

As to the possible causes involved in stimulating cell proliferation and immunoglobulin production in the spleen, two sets of factors must be considered.

Environmental Factors

Owing to the geographical distribution of T.S.S. and the finding of *Plasmodium malariae* in patients with the syndrome in Uganda (Marsden *et al.*, 1965) malaria has been implicated in the aetiology of the syndrome (Pitney, 1968). We found that the incidence of malaria parasitaemia was not different

from that in the general population in Ibadan, and malaria pigment was not seen in liver biopsies from our patients with T.S.S. IgM is the antibody of the primary immune response, and the macroglobulinaemia of trypanosomiasis is attributed to the frequent antigenic variation in that protozoon. In tropical areas the whole population is exposed to innumerable antigens—viral, bacterial, protozoal, and metazoan. Possibly repeated antigenic stimulation by different organisms or antigenic variation of a particular agent giving repeated primary immune responses may play a part in the splenomegaly and macroglobulinaemia of T.S.S.

Though antigenic variation may explain a continued primary immune response it is not clear how this process could protract itself over many decades in a particular person. Also, probably by adulthood an individual would have met most antigens in his environment. Taking into consideration the cross-reactivity of many antigens, one would expect most immune responses at that age to be not of the primary type (with IgM production), but rather of the secondary type (with IgG production). In T.S.S. the IgG level is not significantly different from normal: the abnormality is clearly in the IgM fraction.

Host Factors

It is not yet known whether the macroglobulins of T.S.S. have any protective antibody function, but probably the host is responding in an unusual manner to extraneous antigens, especially as only a few persons in tropical areas develop this syndrome. This finding is increasingly pertinent since a syndrome not unlike T.S.S. has been reported in Britain (Dacie *et al.*, 1969). These host factors, possibly genetic, may initiate or perpetuate the condition, with proliferation of cells in the spleen. The same cells may be producing IgM, and thus account for the serum macroglobulinaemia typical of T.S.S.

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