Immunological Studies in Tropical Splenomegaly Syndrome in Uganda

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Summary: An immunological evaluation carried out in eight patients with the tropical splenomegaly syndrome showed no evidence of impairment in cellular or humoral immunity, though raised levels of macroglobulins were noted in four patients. Hence gross immunological deficiency cannot be associated with the intense lymphoreticular proliferation observed in this disorder.

Introduction

A syndrome of chronic splenomegaly in the tropics associated with lymphocytic infiltration of the hepatic sinusoids (Hamilton *et al.*, 1965) in the absence of other diseases known to produce splenic enlargement has come to be known in Uganda as "big spleen disease" (Marsden *et al.*, 1965; Hamilton *et al.*, 1966) or more widely as the tropical splenomegaly syndrome (Pitney, 1968). A number of investigators have ascribed the condition to an abnormal immunological response to malaria (Gebbie *et al.*, 1964; Marsden *et al.*, 1967; Pryor, 1967). A diligent search for malarial parasites among patients with tropical splenomegaly syndrome in Uganda revealed *Plasmodium malariae* in 45%, as compared with a 4% yield among controls (Marsden *et al.*, 1965). An association with *P. malariae*, however was not shown in similar cases in New Guinea (Marsden *et al.*, 1967). Patients with tropical splenomegaly syndrome have raised antimalaria antibody titres and live in malarious areas of Uganda (Gebbie *et al.*, 1964; Marsden *et al.*, 1965; Hamilton *et al.*, 1965). Dramatic reduction in spleen size following long-term antimalarial prophylaxis has been reported (Watson-Williams *et al.*, 1967; Watson-Williams and Allan, 1968). The virtual absence of haemoglobin AS in patients with tropical splenomegaly syndrome is also strongly suggestive of a malarial aetiology (Hamilton *et al.*, 1969).

If tropical splenomegaly syndrome represents an abnormal immunological host response to malaria an investigation of the general immune competence of the host is needed. This report describes an evaluation of cellular and humoral immune reactivity in eight Ugandan patients with tropical splenomegaly syndrome.

Material and Methods

The patients studied were inpatients at the Lymphoma Treatment Centre and the New Mulago Hospital, Kampala, Uganda, in the period September 1967 to September 1968. The patients were selected on the basis of chronic splenomegaly in the absence of a known cause, and a liver biopsy demonstrating lymphocytic infiltration of the hepatic sinusoids. Liver biopsies were performed with a Menghini needle after appropriate blood coagulation studies, and sections were fixed in formalin and stained with haematoxylin and eosin. Every effort was made to exclude cirrhosis, leukaemia, lymphoma, bacterial infection, and parasitic infestation as aetiological factors.

All investigational procedures were approved by research committees at the National Cancer Institute and Makerere University Medical School, and all patients and controls gave "informed consent" to participate in the study.

Cellular Immunity

Dinitrochlorobenzene (1-chloro-2,4-dinitrobenzene) and five common skin test antigens were used to evaluate the delayed hypersensitivity response. A sensitizing dose of 2,000 μg. of dinitrochlorobenzene in 0·1 ml. of acetone was applied to the medial aspect of the right upper arm within a 2-cm. polyethylene ring, allowed to evaporate, and covered by an adhesive bandage for one week (Brown *et al.*, 1967). Fourteen days after the sensitizing dose 50 μg. and 100 μg. of dinitrochlorobenzene in 0·1 ml. of acetone were similarly applied to separate sites on the right forearm. Challenge tests were read at 48 hours as positive if induration, vesicles, or bullae were present.

The skin tests employed were Brucellergen protein nucleate (Merck Sharp and Dohme), *Candida albicans* extract 1:100 (supplied as dermatophytin O, Holister-Stier Laboratories), mumps antigen (Eli Lilly and Company), intermediate strength purified protein derivative of tuberculin (0·0002 mg. P.P.D.) (Parke Davis & Co.), and trichophyton 1:30 (supplied as dermatophytin, Holister-Stier Laboratories). Skin tests were administered as 0·1 ml. of intradermal injections in the left forearm and read at 48 to 72 hours as positive if greater than 5 cm. of induration was present.

Absolute lymphocyte counts were calculated from at least two white blood counts and differentials. Lymphocyte transformation with phytohaemagglutinin-M (Difco) in vitro was determined by the following procedure.

Heparinized blood 20 ml. was allowed to settle at room temperature for 1 to 2 hours, and the leucocyte-rich plasma was separated into a sterile container. The leucocytes were counted and adjusted to a final concentration of 10^6/ml. with Hyland agammaglobulinaemic newborn calf serum. Then 1 ml. of leucocyte suspension was incubated with 2 ml. of minimal essential media containing 100 units of streptomycin, 100 units of penicillin, and 50 μg. of glutamine per ml. (Flow Laboratories). Cultures were prepared in duplicate with and without 0·05 ml. of phytohaemagglutinin-M and incubated for four days at 37° C. Cultures were harvested by centrifugation at 1,500 r.p.m. for eight minutes in an International Centrifuge No. 269 head, fixed in a freshly prepared mixture of 1:9 glacial acetic acid and 95% alcohol for 10 minutes, and recentrifuged. The cells were pipetted on slides, air-dried, and stained with Giemsa's stain. For each culture 300 cell differential counts of normal lymphocytes, lymphoblastoid lymphocytes, mitoses, and macrophages were performed, and the percentage of trans-

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formed lymphocytes was calculated from the ratio of lymphoblastoid and mitotic cells to the total lymphoid cells counted. Average percentages of the two unstimulated and phytohaemagglutinin-M-stimulated cultures were calculated separately.

Humoral Immunity

Antibody response to Vi antigen§ was measured after the intramuscular administration of 100 μg. of antigen. Pre-immunization and 14-day postimmunization serum was collected, was stored at −20° C., and serum titres were determined in twofold dilutions by a haemagglutination technique (Landy and Lamb, 1953).

Immunoglobulin levels were measured in duplicate by the gel diffusion method of Fahey and McKelvey (1965), Hyland antibody-agar plates being used. Assays were performed at the National Cancer Institute, Bethesda, Md., on specimens of serum collected and stored at −20° C. and shipped frozen from Kampala.

Controls

Twelve men who were admitted to hospital for trauma gave consent to serve as controls for the Vi antibody response and immunoglobulin determinations (courtesy of Mr. John Taylor, Department of Surgery, Mulago Hospital, Kampala, Uganda). Of 20 normal Ugandan children, adolescents, and young adults who gave consent, 19 were successfully sensitized with dinitrochlorobenzene and responded with a typical indurated or vesicular reaction. Lymphocyte transformation with phytohaemagglutinin-M was evaluated in 10 healthy African adults.

Results

Table I shows the clinical features of the patients at the time of testing. The median age was 19 years (range 10–53 years), and there were five males and three females. Splenic enlargement averaged 18 cm. (range 7–32 cm.) below the left costal margin at the mid-clavicular line. Slight liver enlargement was noted in six patients. Lymphocytosis of the hepatic sinusoids

TABLE I.—*Clinical Features of Patients at Time of Study*

Case No.	Age	Sex	Spleen Size*	Liver Size*	Liver Biopsy†	Haemoglobin (g./100 ml.)	W.B.C. (per cu.mm.)	Platelets (per cu.mm.)
1	10	F	20	3	+ +	9·6	4,300	54,000
2	10	F	22	3	+ +	5·6	4,000	61,000
3	15	M	15	2	+ +	12·8	5,200	100,000
4	17	M	7	0	+	13·8	7,000	165,000
5	19	M	9	0	+ +	13·6	3,000	105,000
6	24	M	12	5	+	10·0	6,700	55,000
7	25	F	30	7	+ +	7·3	3,000	153,000
8	53	M	32	4	+ +	7·0	3,500	30,000

* Measured in centimetres below the costal margin in the midclavicular line.
† Sinusoidal lymphocytic infiltrations: + slight; + + moderate.

was slight in two cases and moderate in six. At the time of testing, anaemia was present in five patients and correlated closely with spleen size. Leucopenia (<4,000 W.B.C./cu.mm) was observed in three patients, and platelet counts were reduced to a variable extent in all patients. Absolute lymphocyte counts were normal.

Table II shows the results of the immunological evaluation. In all patients there was at least one positive intradermal test, and seven out of eight patients were sensitized to dinitrochlorobenzene. Lymphocyte transformation to phytohaemagglutinin-M in vitro was normal in every patient. All patients but one had a rise in antibody after immunization with Vi antigen. In Cases 1 and 6 the preimmunization titre was high and may represent previous exposure to the antigen or the presence of cross-reacting antibodies. IgG and IgA values were

§ A polysaccharide isolated from *Escherichia coli* (5396/38), kindly supplied by Dr. M. E. Webster, National Heart Institute, and prepared by Dr. J. F. Gallelei, Clinical Center Pharmacy Department, National Institutes of Health, Bethesda, Md.

TABLE II.—*Immunological Evaluation in Eight Patients with Tropical Splenomegaly Syndrome*

Case No.	Intradermal Tests Positive	D.N.C.B. (100 μg. Reaction)	% Lymphocyte Transformation	Vi Antibody		Immunoglobulin (mg./ml.)		
				Serum Titre	Tube Rise	IgG	IgA	IgM
1	Mumps, P.P.D.	Neg.	74	1 : 64	1	14·6	1·74	7·6
2	Brucella, P.P.D.	Pos.	67	1 : 32	5	15·8	1·75	15·2
3	Mumps, P.P.D.	Pos.	72	1 : 256	4	22·0	2·70	1·86
4	Mumps, P.P.D.	Pos.	70	1 : 512	6	21·0	3·70	1·30
5	Brucella, candidin, mumps, P.P.D.	Pos.	80	1 : 1,024	7	19·4	3·88	1·04
6	Brucella, mumps	Pos.	77	1 : 256	0	33·0	3·75	1·67
7	P.P.D.	Pos.	64	1 : 16	2	13·3	0·89	10·0
8	Brucella, mumps, P.P.D.	Pos.	74	1 : 256	7	18·5	2·2	12·0
Controls (mean ± 1 S.D.)			63 ± 8		5 ± 1·7	18·0 ± 4·8	2·2 ± 1·0	1·0 ± 0·37

comparable to controls, though Case 6 had a markedly raised IgG. IgM values were highly raised in four patients. Serial studies of Ig values in Case 2 before and after splenectomy showed a progressive fall in IgM (15·2 and 0·5 mg./ml.) with a corresponding rise in IgG (15·8 and 22·0 mg./ml.) (Table III).

TABLE III.—*Serial Immunological Studies in Case Two Before and After Splenectomy*

Condition	Immunoglobulin (mg./ml.)		
	IgG	IgA	IgM
Before splenectomy	15·8	1·75	15·2
1 month after splenectomy	21·0	3·28	9·2
6 months after splenectomy	22·0	1·94	0·5

Discussion

Patients with tropical splenomegaly syndrome do not have an associated humoral or cellular immunological disorder as evaluated in this study. Delayed hypersensitivity was intact in each individual as measured by the response to intradermal antigens or to a topically applied allergen (dinitrochlorobenzene). Lymphocyte counts in this group of patients with tropical splenomegaly syndrome were normal, and the circulating lymphocytes transformed normally in vitro following stimulation with phytohaemagglutinin-M.

Six of the eight patients in this study showed normal titre rises to a bacterial antigen (Vi). Two patients (Cases 1 and 6) had existing or cross-reacting antibodies. All patients had normal IgG and IgA levels, and four had raised IgM.

The high levels of IgM in patients with tropical splenomegaly syndrome have previously been reported (Charmot and Vargues, 1963 ; Wells, 1967), and can be distinguished from the macroglobulinaemia of Waldenström (Trincão *et al.*, 1966). The pathogenesis of dysgammaglobulinaemia in tropical splenomegaly syndrome remains obscure, though it is intriguing to speculate that these individuals respond to malarial infection with a predominantly IgM antibody. Several indirect lines of evidence suggest that patients with raised IgM have associated high antimalarial antibody titres (Shaper *et al.*, 1968), and that all the malarial antibody in patients with tropical splenomegaly syndrome resides in the IgM fraction (N. Mody, personal communication). Whether raised IgM levels are associated with an abnormal host response or are related to the nature of the

antigenic stimulus (or both) is the subject of further investigation.

The tropical splenomegaly syndrome or "big spleen disease" of Uganda may be considered as a reactive "lymphoreticular proliferative disorder." The lymphocyte increase is usually confined to the hepatic sinusoids and to the spleen; plasma cells may be present in both organs but are more common in the spleen. Occasionally cases also show an increase of lymphocytes in the upper abdominal lymph nodes (Hutt and Muivah, 1969), in the marrow, and in the blood (Watson-Williams and Allan, 1968). There is some evidence that this "reactive" proliferation of lymphocytes may become neoplastic, with resultant chronic lymphatic leukaemia (Watson-Williams *et al.*, 1967; Lowenthal and Hutt, 1968). Associated with the increase of immunologically competent cells, lymphocytes and plasma cells, in the spleen and liver there is an increase of fixed phagocytic cells (Kupffer cells and splenic histiocytes). Despite this pronounced lymphoreticular proliferation, however, no defect in immunological function was noted in this study.

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Blood Glucose and Insulin Relationships in the Human Mother and Fetus before Onset of Labour

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Summary: The effects of a maternal intravenous glucose load on the fetal plasma levels of glucose and insulin have been studied in 11 patients before the onset of labour. Within five minutes the fetal plasma glucose concentration rose significantly, indicating a rapid transfer of glucose across the placenta. Following this, the rate of fall in fetal plasma glucose closely reflected that in the mother.

Serial fetal insulin estimations carried out in 8 of the 11 subjects following maternal glucose showed an early rise in fetal insulin in four and a delayed rise in one; in the remaining three there was no definite change.

It is concluded that the blood glucose level of the fetus is controlled by that of the mother, but that the fetal pancreas at term may respond to hyperglycaemia by the secretion of insulin.

Introduction

Glucose rapidly crosses the placenta in the presence of a concentration gradient between mother and fetus, but serial recordings of simultaneous fetal and maternal blood glucose levels have seldom been made under conditions of a rapidly altering maternal glucose concentration. Although it is known that the human fetal pancreas contains insulin from the twelfth week of intrauterine life (Steinke and Driscoll, 1965) the extent to which fetal beta cells are capable of responding to metabolic stimuli is uncertain.

Methods

Eleven patients gave informed consent to participate in the investigation. Ten were induced for postmaturity at 42 weeks' gestation. One patient (E) was thought to have a "small for dates" fetus and was therefore induced at 38 weeks. In this patient insulin results only have been included. Apart from

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