non-epidemic rate of the disease is very low. An epidemic began in 1965 and lasted for five years. Unfortunately the serogroup responsible is not known. Compared with that epidemic the short duration of the 1973–74 one is striking. Its decline within one year could not be expected to have occurred by chance, especially since the epidemic was continuing in the population at large, but must be ascribed to the vaccination. Thus vaccination of less than half the new recruits was sufficient to cut short the epidemic spread of the infection. The vaccination status of the trainees (all lots) in service at each time was calculated from the training programmes (fig. 4). The end of the Army epidemic coincides with 36% vaccination.

Discussion

The clinical efficacy and safety of group-A meningococcal vaccines based on the capsular polysaccharides can now be considered established with the studies in schoolchildren in Egypt and the Sudan and our trial in military recruits in Finland (table I). On the basis of confirmed group-A cases we estimate protection to be 89%, and thus similar to results of other trials with group-C or group-A vaccines. We can see no basis for hesitation in the use of this vaccine under epidemic conditions.

Meningococcal disease (group-A) was virtually eliminated from the Finnish Armed Forces after vaccination began. Consequently, it was decided to vaccinate all new recruits in 1975, and this has been done with the accidental exception of a few hundred men. During the first six months of service of the 1975 recruits, 1 group-A case has been seen in a vaccinated man, and there was another case in one small garrison where vaccination had not been done. The United States Army started routine use of group-C vaccine in 1971, resulting in a very striking reduction of group-C disease.[13]

Our findings (fig. 4) suggest that considerably less than 100% vaccination is sufficient to stop the epidemic. This situation may be peculiar to meningococcal epidemics caused by group-A organisms. It is tempting to relate this finding with the mode of spread of meningococcal infection, which seems to require close and frequent contacts between individuals.

Meningococcal infection apparently spreads as a respiratory infection in which nasopharyngeal carriage is important. Meningococci are normal constituents of the nasopharyngeal flora of man, and we found that 35% of the recruits carried meningococci on arrival. Close contacts such as prevail in military quarters promote spread of infection and lead to higher carrier frequencies.[14 15] After one month of service the carrier-rate had risen to 54%. In contrast, the epidemic strain—which could be identified by its sulphonamide resistance and its serological group-A property, both unusual among local meningococci—was rare in the nasopharyngeal samples, being 1·5% on arrival and 3·2% after one month. We feel that the high incidence of disease and low carrier-rates speak for the virulence of the strain causing the epidemic. The low carrier-rate also means that development of immunity in the population will be slow, and that the epidemic could last a long time in the absence of specific intervention.

We thank Merck Sharp & Dohme Research Laboratories for the vaccine, the medical staff of the Finnish Armed Forces for their co-operation, Dr M. Artenstein and Dr M. Hilleman for advice, the personnel in our laboratories for help, and Merck Sharp & Dohme Research Laboratories, the National Medical Board of Finland, and the Sigrid Jusélius Foundation for financial support.

Requests for reprints should be addressed to P.H.M., Central Public Health Laboratory, Mannerheimintie 166, 00280 Helsinki 28, Finland.

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CHRONIC LIVER DISEASE DEVELOPING AFTER OUTBREAK OF HBsAg-NEGATIVE HEPATITIS IN HÆMODIALYSIS UNIT

R. M. GALBRAITH
A. L. W. F. EDDLESTON
B. PORTMANN
ROGER WILLIAMS

King's College Hospital, London SE5 8RX

P. E. GOWER

Charing Cross Hospital, London W6 8RF

Summary Chronic liver disease developing after acute hepatitis type B is well documented, but is not thought to occur after acute hepatitis due to other viruses. However, follow-up of 29 patients in a hæmodialysis unit who contracted HBsAg-negative acute hepatitis during 1968-70 revealed 8 cases with raised serum-aminotransferase levels dating from that time. Liver biopsy in 7 of these disclosed chronic aggressive hepatitis in 3, of whom 2 had already progressed to advanced cirrhosis. Chronic persistent hepatitis was present in 2 others, and the remaining 2 had non-specific hepatitis in association with massive iron overload. Immunological studies demonstrated a higher frequency of cellular immunity to HBsAg in those who had previously had acute hepatitis than in those who had not, although the prevalence of humoral antibody was similar in the two groups. One possible explanation for these findings is the presence of immunological cross-reaction at a cellular level between the hepatitis B virus and that responsible for the initial outbreak.

Introduction

OUTBREAKS of acute hepatitis in hæmodialysis units affecting both patients and staff have given much cause for concern. The two outbreaks in the renal unit at Charing Cross Hospital in 1966-67[1] and 1968-70[2] were

unusual in that the hepatitis B surface antigen (HBsAg) was never detected even by radioimmunoassay and the clinical illness was mild. On the basis of this and other epidemiological findings, the hepatitis A virus was presumed to be the responsible agent. Although chronic liver disease is well documented after acute HBsAg-positive hepatitis in patients with renal disease[3] as well as in previously healthy individuals,[4] it is very rare after HBsAg-negative hepatitis. We describe here the immunological and histological abnormalities found in 8 of the Charing Cross Hospital patients who have had persistently raised serum-aminotransferase levels since the time of the second outbreak.

Patients and Methods

The second outbreak of acute hepatitis has been described by Coleman et al.[2] All patients had previously had normal liver-function tests and bromsulphthalein retention. Tests for HBsAg, including radioimmunoassay, were repeatedly negative, and other causes, such as toxoplasmosis, cytomegalovirus, and the Epstein-Barr virus were excluded. Of the 68 patients who were on maintenance dialysis during the outbreak, 29 had acute hepatitis. 7 of these 29 have since died from causes other than liver disease, 15 have been maintained on hæmodialysis, and 7 have functioning renal transplants. Of the 39 patients who did not contract acute hepatitis, 10 have died, 18 have remained on hæmodialysis, and 11 have had a successful transplant.

After the outbreak, patients were screened regularly, standard biochemical tests of liver function including bilirubin, aspartate and alanine aminotransferases (A.S.T., A.L.T.), alkaline phosphatase, albumin, and globulin being done every three months and more frequently if abnormal. Those with abnormalities on two or more occasions were further investigated—prothrombin-time, serum-protein electrophoresis and serum-immunoglobulin levels, serum autoantibodies, liver scan, and, most recently, needle biopsy. Sera were examined for HBsAg by a solid-phase radioimmunoassay which was also used to detect antibody to the surface antigen (anti-HBs).[5] Cellular immunity was investigated using the leucocyte migration test[6] with HBsAg as antigen,[7] the normal range for migration indices being 0·80–1·20.

Results

Of the 15 patients who had remained on hæmodialysis after developing acute hepatitis, 8 have had persistently abnormal A.S.T. levels since their initial illness (fig. 1).

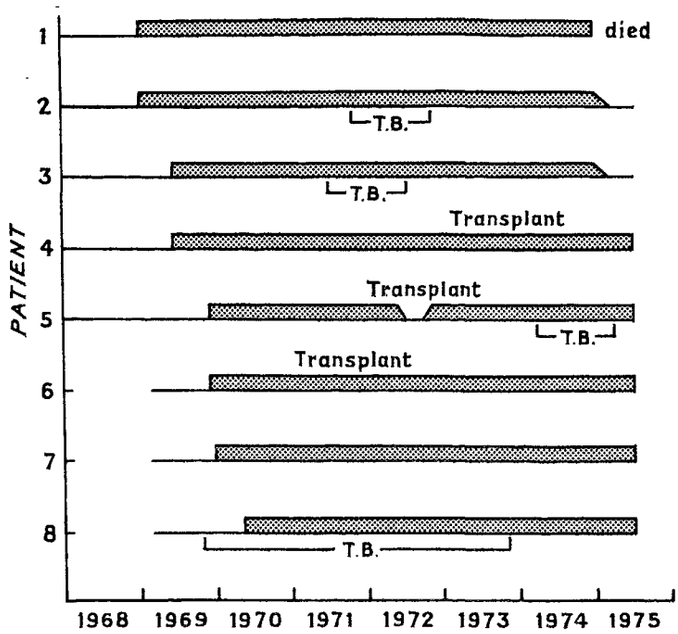

Fig. 1—Clinical course in 8 hæmodialysis patients with persistently raised A.S.T. levels (solid bars).

T.B. represents antituberculous therapy. Transplants were performed in case 4 (1973), case 5 (1972), and case 6 (1971) but were removed after 2, 8, and 2 months respectively.

1 of these (case 5) received a kidney transplant in 1972, two years after contracting acute hepatitis, and for the next eight months, while he was taking prednisolone and azathioprine, his serum-A.S.T. returned to normal. However, after rejection and removal of the graft, the A.S.T. rose again. 2 other grafts (in cases 4 and 6) never functioned satisfactorily, and were removed shortly after insertion; the A.S.T. levels remained high. Patients 2, 3, 5, and 8 had received antituberculous therapy for suspected tuberculosis, but the diagnosis was confirmed only in case 8, where liver biopsy in 1971 had revealed multiple granulomas and acid-fast bacilli on Ziehl-Neelsen staining. Furthermore, the raised transaminases could not be related temporally to the antituberculous therapy because they were recorded before treatment began in

TABLE I—MAIN FINDINGS IN THE 8 PATIENTS WITH ELEVATED SERUM AMINOTRANSFERASE LEVELS

Case	Sex	Age	Hepatomegaly (cm)	Splenomegaly (cm)	Serum-bilirubin (μmol/l)*	Maximum A.S.T. (I.U./l)†	Serum-albumin (g/l)	Liver histology
1	M	54	4	2	19	125	25	Chronic aggressive hepatitis; with cirrhosis
2	M	53	8	0	35	150	35	Chronic aggressive hepatitis; with cirrhosis
3	M	54	2	0	16	100	45	Chronic aggressive hepatitis
4	M	35	2	0	8	100	38	Chronic persistent hepatitis; granuloma; grade-II siderosis
5	M	26	2	0	10	95	45	Chronic persistent hepatitis; granuloma
6	F	47	2	0	12	100	36	Grade-IV siderosis; non-specific hepatitis; granuloma
7	M	43	0	0	8	90	35	Grade-IV siderosis; non-specific hepatitis; granuloma
8	M	37	4	2	8	120	40	Not available

* Normal <20 μmol/l. † Normal <50 I.U./l.

cases 2, 3, and 5 and persisted after treatment in case 8 for more than a year (fig. 1).

Liver biopsies done within the past nine months show that 3 of these 8 patients have developed the typical histological changes of chronic aggressive hepatitis. The liver was enlarged on clinical examination, and in case 1 the spleen was also palpable (table I), this patient showing signs of hepatocellular failure with ascites and a low serum-albumin. All 3 patients had moderately raised serum-alkaline-phosphatase levels, and electrophoresis showed the enzyme increase to be of hepatic origin. Abnormalities in standard liver-function tests were otherwise slight, the serum-bilirubin being raised in only 1 patient. The prothrombin-time and serum-globulin were normal in all 3 and the A.S.T. never rose above 150 I.U./l (table I).

2 of these 3 patients (cases 2 and 3) had had a liver biopsy in 1972, during investigations for suspected tuberculosis, and chronic persistent hepatitis had been reported. However, we thought that the changes in case 2 were consistent with chronic aggressive hepatitis (fig. 2a). The later biopsy in this patient revealed a similar picture of inflammatory infiltration and cell damage but there was also definite cirrhosis (fig. 2b). In case 3, the lesion has also progressed with development of chronic aggressive hepatitis but not, as yet, of cirrhosis. Both of these patients were started on immunosuppressive therapy (prednisolone 15 mg and azathioprine 75 mg daily) and have shown symptomatic and biochemical improvement. Case 1, however, deteriorated during the six months after these investigations and died in advanced liver failure. At necropsy, the liver was shrunken and grossly cirrhotic with large regeneration nodules.

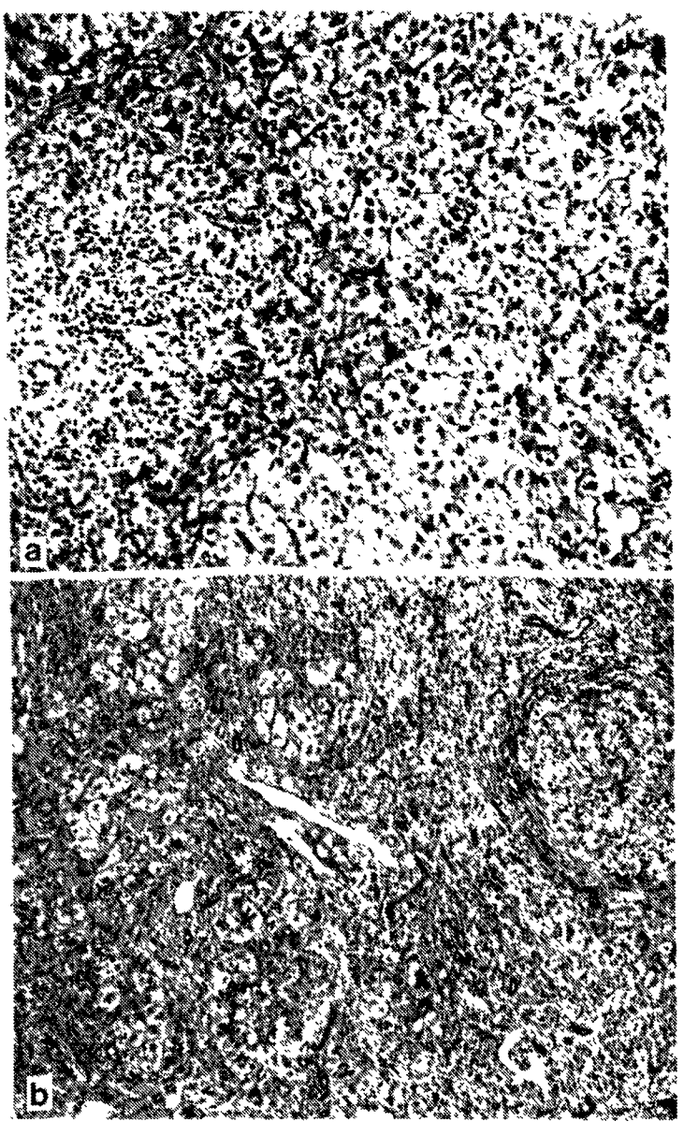

Fig. 2—Histological findings in case 2.

(a) Chronic aggressive hepatitis: 1972.
(b) Chronic aggressive hepatitis and cirrhosis: 1974.
(Hæmatoxylin and eosin; reduced to 2/3 of ×90.)

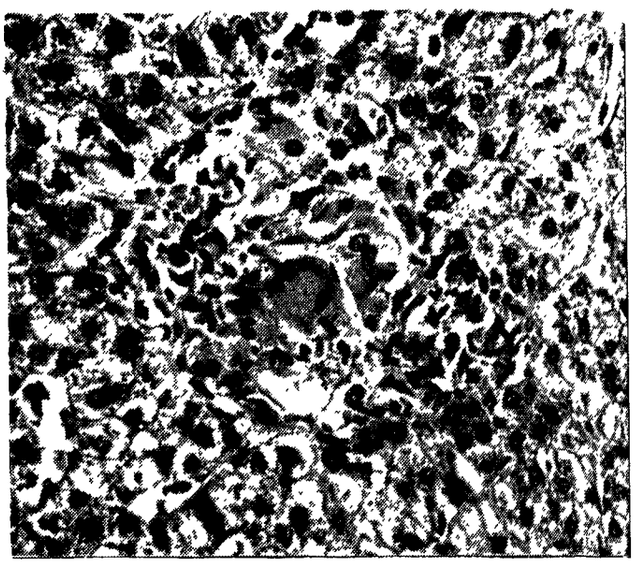

Fig. 3—Non-caseating granulomas.

(Hæmatoxylin and eosin; reduced to 2/3 of ×375.)

Liver biopsy was also done in 4 of the other 5 patients who had persistently raised serum-A.S.T. levels. In two, both of whom had hepatomegaly on clinical examination, the changes were those of chronic persistent hepatitis. The other two had grade-IV siderosis according to the criteria of Scheuer et al.,[8] together with mild non-specific hepatitis. In all 4, non-caseating granulomas were also seen (fig. 3), but no clinical evidence of tuberculosis was found and no mycobacteria could be demonstrated on Ziehl-Neelsen staining. In none of these biopsy samples nor in those showing chronic aggressive hepatitis were any hepatocytes found to contain HBsAg, using either the orcein stain or specific immunofluorescence. The remaining patient (case 8), who has had moderate hepatosplenomegaly in association with his raised serum-A.S.T. for several years, has refused further investigation.

We found no clinical or biochemical evidence of chronic liver disease in the 7 patients who had contracted acute hepatitis during the outbreak, but in whom a functioning renal transplant had subsequently been established. This also applied to the 39 patients who had not contracted acute hepatitis and who were being maintained by hæmodialysis or by a renal transplant.

The results of immunological tests in the 8 patients with persistently raised serum-A.S.T. levels were compared with those obtained in the other 7 who had developed acute hepatitis but had shown no subsequent hepatic abnormalities (table II). Also shown in table II are the findings in the 18 patients who were also on hæmodialysis but who had not contracted acute hepatitis during the outbreak. Serum levels of IgG and IgM were raised in some of the patients, but the rises were small except in two, with IgM levels of greater than 5·8 g/l (upper limit of normal 1·80). Autoantibodies were detected in

TABLE II—FREQUENCY (%) OF IMMUNOLOGICAL ABNORMALITIES IN PATIENTS REMAINING ON HAEMODIALYSIS

Clinical groups	No.	IgG (>16·0g/l)	IgM (>1·8g/l)	Autoantibodies		Anti-HBs	L.M.T. to HBsAg
				S.M.A.	A.N.A.		
Previous acute hepatitis							
A.S.T. increased	8	50	50	13	0	25	88
A.S.T. normal	7	0	57	0	14	29	71
No previous acute hepatitis	18	17	22	22	22	33	33

A.S.T. = Aspartate aminotransferase
S.M.A. = Smooth-muscle antibody
A.N.A. = Antinuclear antibody
L.M.T. = Leucocyte-migration test

the serum of several patients, but in no case did the titre exceed 1/10. However, no significant difference could be found between the three groups of patients with respect to the frequency either of abnormal immunoglobulin levels or of serum autoantibodies. The frequency with which anti-HBs was found in the serum was also very similar—25%, 29%, and 33% respectively. However, the frequency of positive leucocyte-migration tests with HBsAg was significantly higher in the 15 patients who had contracted acute hepatitis (whether or not chronic liver disease subsequently developed) than in the 18 who had not ($P<0·025$).

Discussion

The development of chronic liver disease after acute hepatitis type B has been reported both in patients who remain HBsAg-positive and in those who successfully eliminate the antigen. In the Copenhagen study, 10 of 182 patients with acute hepatitis type B remained HBsAg-positive, liver biopsy revealing chronic aggressive hepatitis in 8 and chronic persistent hepatitis in 2. A similar evolution was also observed in 6 of the 47 in whom HBsAg had been cleared from the serum.[4] In contrast, progression to chronic liver disease after acute hepatitis type A is very rare. Indeed, Gilon has found no clinical or biochemical evidence of chronic hepatic dysfunction in over 2000 patients followed up for between one and ten years.[9]

We found biochemical evidence of continuing liver disease in 8 (53%) of the 15 patients maintained on hæmodialysis who had contracted acute hepatitis during the outbreak. Similar elevations in serum-A.S.T. levels were not observed in the other patients with acute hepatitis who had a satisfactorily functioning kidney transplant. The reason may lie partly in the immunosuppressive drugs they were receiving. Such therapy would be expected to attenuate biochemical abnormalities, as indeed happened in 1 of the patients with chronic persistent hepatitis during the six-month period in which he had a functioning transplant. However, the decision to do a liver biopsy was determined by the finding of a persistently raised aminotransferase, so cases of liver disease in the transplanted group may have been missed. Certainly chronic aggressive hepatitis can occur in renal-transplant recipients, and Freiberger et al. report its occurrence in 3 of 30 such patients, 1 of whom died in liver failure.[10] An additional factor in this situation is azathioprine, which may cause liver damage by a direct hepatotoxic effect,[10 11] although this is only well established in animals.

Despite the severity of the histological changes in the 3 patients with chronic aggressive hepatitis, abnormalities in liver-function tests (especially A.S.T. and serum-globulin) were surprisingly mild. This may be related to the known immunosuppressive effects of chronic renal failure, even when patients are apparently well maintained on hæmodialysis. Nevertheless, the prognosis of chronic aggressive hepatitis in this situation is poor, as shown by the patient in whom progressive liver failure rapidly developed. The 2 patients with chronic persistent hepatitis are not currently being treated with prednisolone but will be watched closely in case a more aggressive lesion develops, as happened in case 3.

The mild non-specific hepatitis seen in 2 of the patients can be attributed to massive iron overload, but the cause of the non-caseating granulomas was not established. In particular, no evidence of tuberculosis was found. One possible cause is a foreign-body tissue reaction resulting from emboli of normally non-pathogenic microorganisms or of biologically inert material entering the circulation during peritoneal or hæmodialysis. This could also explain the transient opacities which had been observed on chest X-ray in several of these patients.

The high prevalence of anti-HBs in these patients (30% as compared with 7% in healthy controls) probably reflects the greater exposure to hepatitis B virus, and is very similar to that reported in healthy laboratory staff who handle HBsAg-positive material.[7] In such staff, the frequency of cellular immunity to HBsAg (90%) is also much higher than in normal controls (30%), and a correspondingly high figure would therefore be expected in renal patients. However, this was so only in those with a previous history of acute hepatitis, and the cause of this dissociation between cellular and humoral immunity is not clear. Exposure to the hepatitis B virus appears to be similar in the two groups since the number of hospital admissions and of blood-transfusions were no different. Selective depression of cellular immunity may have been present in those in whom there has been no evidence of hepatitis. However, proof of this would depend on a knowledge of the frequency of sensitisation to HBsAg in the two groups before the outbreak. An alternative possibility is that the high frequency of cellular immunity to HBsAg in the patients who contracted acute hepatitis may be due to immunological cross-reaction between the hepatitis B virus and that responsible for the 1968-70 outbreak.

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Requests for reprints should be addressed to R. W.

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(References continued overleaf)

CHRONIC LYMPHOCYTIC LEUKÆMIA OF T-CELL ORIGIN

IMMUNOLOGICAL AND CLINICAL EVALUATION IN ELEVEN PATIENTS

JEAN-CLAUDE BROUET GEORGES FLANDRIN
MARILYNE SASPORTES JEAN-LOUIS PREUD'HOMME
MAXIME SELIGMANN

Laboratories of Immunochemistry and Immunopathology (INSERM U. 108) and Cytology and Immunohæmatology (INSERM U. 93), Research Institute on Blood Diseases, Hôpital Saint-Louis, 75475 Paris Cedex 10, France

Summary Eleven patients with chronic lymphocytic leukæmia of T-cell origin are reported. The identification of the leukæmic cells was performed with seven different membrane markers for either T or B lymphocytes. The reactivity of the leukæmic T cells with three different heteroantisera to T cells differed from patient to patient but was homogeneous in individual cases. This finding suggests that the leukæmic lymphocytes belonged to a single subset of T cells. These lymphocytes responded to allogeneic cells in some of these patients. In contrast, stimulation by non-specfic mitogens was poor in most patients. Two patients were affected with the prolymphocytic type of chronic lymphocytic leukæmia, but a characteristic clinical and hæmatological pattern was found in nine patients. The blood and marrow infiltration was moderate and the proliferating T lymphocytes had a high content of lysosomal enyzmes in all patients and cytoplasmic granules in six cases. Other unusual features included massive splenomegaly (five patients), skin lesions (four patients), and major neutropenia (four patients).

Introduction

NEOPLASTIC B-cell diseases are more common in man than T-cell proliferations,[1] such as Sézary syndrome[2 3] and 30% of cases of common acute lymphoblastic leukæmias.[4–7]

Chronic lymphocytic leukæmia (C.L.L.) of T-cell origin is considered to be a rare disorder. We found three such cases among more than one hundred and fifty C.L.L. patients[8] and only six other cases have been reported[9–14] while hundreds of cases of C.L.L. have been screened for various T and B lymphocyte surface markers. Our present series of eleven patients, ten of whom attended a single department of hæmatology, suggests that this disorder may not be so rare. Because it is characterised by peculiar hæmatological and clinical features, it may have been overlooked and not included in previous C.L.L. series. The immunological characterisation of the proliferating cells by several antisera to T cells revealed striking differences from patient to patient, contrasting with an homogeneous reactivity of all the proliferating cells in individual cases. This finding strongly suggests that the leukæmic cells arise from only one T-cell subpopulation.

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Methods

Cytological Studies

Peripheral blood and bone-marrow smears were stained with May-Grünwald-Giemsa, and periodic-acid/Schiff and for peroxidase, monocyte-specific esterase, β-glucuronidase, and acid-phosphatase activity by the usual cytochemical techniques.[15 16] For electron microscopy, buffy-coat pellets were fixed in glutaraldehyde, post-fixed in 1·25% osmium tetroxide for 30 min, embedded in 'Epon 812', and stained with uranyl acetate.

Immunological Studies

The procedures used for the separation of blood lymphocytes or bone-marrow white cells, and for the detection of surface immunoglobulin (Ig) by staining living cells in suspension in the cold have previously been described.[17] In one case, a skin nodule was excised and gently teased to liberate infiltrating cells.

Surface Ig were studied after short-term in-vitro culture following trypsinisation to investigate the actual synthesis of surface Ig by the lymphocytes.[18] Fluorescein-conjugated aggregated IgG were used at a concentration of 1–2 mg/ml in order to detect the "receptor" for the Fc fragment of IgG.[18 19]

Spontaneous rosette-forming cells were detected according to the technique described by Jondal et al.[20]

The preparation of rabbit heteroantisera to peripheral-blood T cells from a boy with X-linked agammaglobulinæmia and virtual absence of B cells (anti-X.L.A. serum), to fetal thymocytes (anti-F.T. serum), to human fetal brain, and to C.L.L. B cells have been described in detail elsewhere.[5 21] Briefly these antisera were heat inactivated, absorbed on human AB erythrocytes (×3) human liver (×3), and on normal human serum, IgG, IgA, and IgM coupled to 'Sepharose 4 B'. They were further absorbed on suitable B or T cells to make them specific by either cytotoxicity or indirect immunofluorescence. By cytotoxicity, with an end-point of 1/16, anti-F.T. and anti-X.L.A. sera killed, respectively, 60% and 75%, and anti-C.L.L. sera less than 20% of normal peripheral-blood lymphocytes. About half of tonsil lymphocytes were killed by all these sera. Anti-T sera gave negative results on B C.L.L. cells as did anti-b sera on Sézary cells of T-derived blast cells from A.L.L.

An indirect fluorescence method was used in some cases with a second layer of rhodamine-conjugated IgG purified from a sheep antiserum to rabbit Ig. Double labelling experiments with fluorescein-conjugated IgG aggregates or with goat antiserum to human Ig were performed in order to control the specificity of the various antisera to B or T cells on normal blood lymphocytes. The specificity of these sera by immunofluorescence was further assessed on cultured lymphoblastoid cell lines of known T or B origin and on B C.L.L. cells. The antiserum to brain stained only 30% of blood T lymphocytes and was negative on thymocytes.[21]

The peripheral-blood lymphocytes of the patients were stimulated by non-specific mitogens, phytohæmagglutinin or pokeweed, or allogeneic cells (mixed lymphocyte reaction, M.L.R.)[22] For experiments with non-specific mitogens the results of thymidine uptake were recorded at 72 h and expressed as percentage of the control for a standard dilution (1/100 final) of both mitogens. M.L.R. response and M.L.R.-stimulating ability are recorded as "normal", "low", or "zero", as compared with the two normal controls included in each test.

Results

Clinical Data (table I)

The past history of the patients was unremarkable