Antibodies to Epstein-Barr and other viruses in children with acute lymphoblastic leukaemia

R. N. P. SUTTON, S. D. MARSTON, H. J. M. PULLEN, C. W. DARBY, D. I. K. EVANS, and R. T. D. EMOND

From the Department of Medical Microbiology, King's College Hospital Medical School, the Department of Haematology, Institute of Child Health, The Hospital for Sick Children, and the Royal Free Hospital Infectious Diseases Unit, Coppelts Wood Hospital, London; and the Department of Haematology, Royal Manchester Children's Hospital

Sutton, R. N. P., Marston, S. D., Pullen, H. J. M., Darby, C. W., Evans, D. I. K., and Emond, R. T. D. (1974). *Archives of Disease in Childhood*, 49, 540. **Antibodies to Epstein-Barr and other viruses in children with acute lymphoblastic leukaemia.** Sixty-eight children with acute lymphoblastic leukaemia were tested for antibodies to Epstein-Barr (EB) virus and to other viruses. The antibody response to the test viruses in these children was unremarkable, with the exception of EB virus, where the presence of complement-fixing antibodies in children tested during the first month of their illness suggested that a higher proportion had previous experience with this virus than was the case in control children. The implications of this observation with regard to leukaemogenesis are discussed.

The possibility that infectious mononucleosis might be a self-limited form of leukaemia has been raised on a number of occasions (Dameshek and Gunz, 1964). This speculation has not been supported by studies with the EB virus, the causative agent of infectious mononucleosis, and infections with this virus are not uncommon during the course of acute leukaemia (Stevens *et al.*, 1971). Nevertheless, the relation of the EB virus to malignant disease and recent reports of infectious mononucleosis being followed closely by leukaemia (Levine *et al.*, 1972) prompted further investigation in this field. Our observations (Sutton *et al.*, 1973) that in infectious mononucleosis antibodies to a soluble complement-fixing antigen specified by the EB virus develop much later than antibodies to the EB virus capsid antigen (from which it is quite distinct—Marston *et al.*, 1972) allow a somewhat more extensive interpretation to be made of serological results than that based upon the estimation of one type of antibody only.

We present the results of investigations in children with acute lymphoblastic leukaemia and with other conditions. The normal responses to infections with common viruses indicate that the

humoral immune responses of these patients remained intact, and therefore allow confidence to be placed in the estimates of EB virus antibodies.

Methods

Sera were obtained from children admitted to hospital with acute lymphoblastic leukaemia, with infectious mononucleosis, and with other conditions. In those with leukaemia the criteria for cytological diagnosis were those of Hayhoe, Quaglino, and Doll (1964); in those with infectious mononucleosis criteria for diagnosis were clinical, together with the presence of heterophile antibodies and typical abnormal cells in the peripheral blood. Children with conditions other than leukaemia or infectious mononucleosis formed a control group. These conditions included infectious diseases (e.g. typhoid fever, viral meningitis), congenital abnormalities (e.g. congenital heart disease, microcephaly), and miscellaneous conditions (e.g. sickle-cell anaemia, polymyositis). Sera from these control children were matched for age with those from the leukaemic patients.

Laboratory methods. Antigens and control sera were provided by the Standards Laboratory, Central Public Health Laboratory, London, except for rubella haemagglutinating and cytomegalovirus and EB virus complement-fixing (CF) antigens which were prepared and assayed by methods described elsewhere (Sutton, Marston, and Emond, 1971b; Sutton, Darby, and

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Gumpel, 1971a). Rubella haemagglutination inhibiting antibodies were estimated by the method of Stewart *et al.* (1967). Antibodies to EB virus capsid antigen were assayed by an immunofluorescent method (Sutton *et al.*, 1973). Immunoglobulins were assayed by a single diffusion precipitin method and hepatitis B antigen was detected by double diffusion in agar gel, using a micro-Ouchterlony technique. All sera were coded and tested in ignorance of their source and were stored without preservative at -20°C .

Results

The population under study comprised 68 children (mean age 4.5 years) with acute lymphoblastic leukaemia seen between April 1965 and November 1970. 41 were tested on only one occasion; 27 were observed for periods of up to 37 months (mean 18.5 months), and from each of these patients from 2 to 12 specimens of serum were obtained, often at about 2-monthly intervals. 172 sera were obtained in this way; small volumes prevented each test from being applied to every specimen.

A second group (mean age 7.6 years) included 22 children who were admitted to hospital with infectious mononucleosis and tested within 1 month of onset and 12 who were tested in convalescence (1 to 14 months, mean 3 months, after onset). The control group comprised 56 children (mean age 4.4 years) with conditions other than leukaemia or infectious mononucleosis.

Development of antibodies to EB virus. Antibodies to EB virus capsid antigen were present in 14 of 28 leukaemic children and in 16 of 31 control

patients ($P < 0.05$), indicating identical rates of infection but giving no indication as to when such infections could have occurred. 125 sera from 58 leukaemic children were tested for EB CF antibodies. 22 patients were tested more than once, of whom 3 had no detectable antibody and 12 had low levels (1/16 or less) in their initial serum specimen. Of these 15 patients, 2 (13%) subsequently developed fourfold or greater increases in antibody; in neither case were there any remarkable clinical features associated with this serological conversion.

EB CF antibody levels in sera which had been taken from 10 children within 1 month of the onset of leukaemia were compared (Table and Fig.) with those in 39 control patients, in 22 children with acute infectious mononucleosis who were tested within 1 month of onset, and in 12 children who were tested during convalescence after infectious mononucleosis (mean 3 months after onset). Infectious mononucleosis in the past is commonly associated with EB CF antibody levels of greater than about 1/16, and the results in the leukaemic children resembled those in the group with known past infectious mononucleosis and differed significantly from the control group and the group with active infectious mononucleosis.

Development of antibodies to other viruses. The prevalence of cytomegalovirus antibodies in 65 sera from leukaemic patients (17%) resembled that observed by Stern and Elek (1965) in healthy children of the same ages. 6 of 25 seronegative patients developed cytomegalovirus antibodies; in 3,

TABLE
EB CF antibodies in children under 16 years of age

	Patients with active infectious mononucleosis tested within 1 mth of onset	Patients with past history of infectious mononucleosis (mean 3 mth after onset)	Patients with acute leukaemia tested within 1 mth of onset	Control patients (same ages as leukaemic patients)
Mean age (yr)	8.6	11.5	4.9	4.8
EB CF antibody levels				
> 1/16	6	8	9	18
≤ 1/16	16	4	1	21
Significance of differences between groups				
Against patients with active infectious mononucleosis	—	$P = 0.047^*$	$P = 0.0013^*$	$\chi^2 = 1.365$ $P > 0.2 < 0.3$ (NS)
Against patients with past history of infectious mononucleosis	—	—	$P = 0.19^*$ (NS)	$P = 0.18^*$ (NS)
Against control patients	—	—	$P = 0.012^*$	—

*Fisher's exact probability test.

FIG.—EB virus complement-fixing antibody levels in patients with infectious mononucleosis, acute lymphoblastic leukaemia, and other conditions.

serological conversion was asymptomatic. The prevalence of adenovirus antibodies in 65 sera from leukaemic patients (65%) resembled that in 76 nonleukaemic hospital patients of the same ages (64%). In 7 of 24 patients there was evidence of adenovirus infection, either asymptomatic or associated with mild respiratory illnesses. 4 leukaemic children had measles, 3 had rubella, and 2 had varicella (all confirmed virologically). These infections were uneventful with the exception of a girl of 5 years who succumbed to fulminating haemorrhagic varicella.

Hepatitis B antigen was present in 3 of 121 sera from 41 leukaemic children. 26 patients were tested on more than one occasion and 3 became positive 3, 10, and 27 months after the onset of leukaemia; in 1 child, reversion to a negative state occurred. 1 child developed jaundice and ascites shortly after the detection of hepatitis B antigen and in the other 2 infection was apparently asymptomatic.

As a general index of antibody production, total IgM, IgA, and IgG immunoglobulins were estimated in 12 episodes of infection in leukaemic children: low IgA levels (less than 10% of Medical Research Council standard serum) were observed in 2 patients during cytomegalovirus and adenovirus infections. In 2 other patients, serial tests over about 12 months showed infections with adenovirus and with cytomegalovirus; IgM, IgA, and IgG immunoglobulins were not depressed over this

period and there was no evidence that these infections were associated with any humoral immunological deficiency.

Discussion

The development of hepatitis B antigenaemia and of EB CF antibodies during the course of leukaemia was probably related to blood transfusion (Gerber *et al.*, 1969; Kapsenberg *et al.*, 1970); the presence of hepatitis B antigen in 7.5% of the leukaemic children underlines the potential risks of sera from these patients. Otherwise, the leukaemic children responded to viral infections in a normal fashion.

The presence of EB CF antibodies in children tested within 1 month of the onset of leukaemia was a surprising observation. Their presence was probably not simply related to the effects of lymphoproliferation, for no differences were apparent in incidence or levels of antibodies to EB virus capsid antigen.

EB CF antibodies, which differ in several respects from EB virus capsid antigen antibodies (Marston *et al.*, 1972), develop regularly after infectious mononucleosis. These CF antibodies develop slowly and, though sometimes detectable in individuals with active infectious mononucleosis, are normally only detectable after some months. Young children, aged 5 years and under, respond to infection with EB virus in much the same way as adults (Sutton *et al.*, 1974), though in this age group heterophile antibodies are observed only rarely (Vahlquist, Ekelund, and Tveteras, 1958).

Leukaemic patients often receive blood transfusions, and infection with EB virus may be acquired in this way. However, high levels of EB CF antibody are not found in patients with infectious mononucleosis tested within 1 month of onset and this suggests that the high levels observed in our leukaemic patients tested within 1 month of onset were not associated with such blood transfusions. Indeed, in the leukaemic children tested during the first month of illness, the proportion with CF antibodies resembled that in controls with past infectious mononucleosis who had been tested in convalescence. This suggests that they experienced an asymptomatic EB virus infection some months before their diagnosis. Some recent reports indicate that this may be related to the development of their leukaemia.

The herpes-like EB virus (a DNA virus) is associated with Burkitt's lymphoma (Henle and Henle, 1966), with nasopharyngeal carcinoma (zur Hausen *et al.*, 1970), with Hodgkin's disease (Johannsson *et al.*, 1970), and with infectious mononucleosis (Henle, Henle, and Diehl, 1968;

Evans, Niederman, and McCollum, 1968). However, nucleic acid homologous with that of the RNA Rauscher murine leukaemia virus has been detected in Burkitt's lymphoma and nasopharyngeal carcinoma cells (Kufe, Hehlmann, and Spiegelman, 1973) and in Hodgkin's disease (Hehlmann, Kufe, and Spiegelman, 1972a). It has also been detected in leukaemic cells (Hehlmann, Kufe, and Spiegelman, 1972b) and antigenic relations have been observed (Mann, Halterman, and Leventhal, 1973) between acute leukaemic cells and cells infected with Rauscher leukaemia virus. Patients with infectious mononucleosis have been known to develop Hodgkin's disease (English, 1970), Burkitt's lymphoma (Cohen *et al.*, 1970), and acute leukaemia (Levine *et al.*, 1972).

Thus, we have evidence for some kind of link between infectious mononucleosis and malignant disease (if on rare occasions) and apparently conflicting evidence which associates both DNA and RNA viruses with these conditions.

These conflicting reports may be reconciled when we consider the ability of the EB virus in infectious mononucleosis to induce a temporary depression of cell-mediated immunity, as indicated by the response to tuberculin (Haider *et al.*, 1973). This depression of cell-mediated immunity (either as here suggested by EB virus, or by other means) could well be the necessary stimulus required to activate otherwise dormant RNA tumour virus genomes, such as those whose vertical transmission has been shown in mice by Huebner and his colleagues (1970). Alternatively, some other facet of the complex immunopathology induced by EB virus infection (Lancet, 1973) might be involved. In a different, but analogous, experimental situation such an activation of an endogenous, vertically transmitted, RNA tumour virus by a DNA virus has been demonstrated by Rhim and Huebner (1973). In man, some indirect evidence exists for the presence of such a vertically transmitted agent, in that raised IgM levels have been detected in neonatal sibs of children with leukaemia (Sutton, Bishun, and Soothill, 1969; Chandra, 1972).

Our observations, though statistically significant, are based upon few patients and their interpretation is speculative, but we consider that they provide a working hypothesis and some facts on which further investigations of the aetiology of acute leukaemia may be planned.

We are grateful to Professor J. A. Dudgeon, Department of Microbiology, Institute of Child Health, London, in whose department some of this work was carried out; to Sir Christopher Andrewes and Professors A. C. Cunliffe and R. M. Hardisty for advice; to Dr.

Hillas Smith; and to the consultants of The Hospital for Sick Children, Great Ormond Street, the Royal Manchester Children's Hospital, and King's College Hospital for permission to investigate patients. We are indebted to Dr. C. P. Bradstreet, Standards Laboratory, Central Public Health Laboratory, for provision of reagents; to Mrs. E. J. P. Almond, Mr. G. T. Hawkins, and Mrs. K. Reynolds for invaluable help; and to Professor J. F. Soothill, Department of Immunology, Institute of Child Health, for carrying out the immunoglobulin estimations. Financial support was provided by the Research Committee, King's College Hospital, the Cancer Research Campaign, and the Leukaemia Research Fund.

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Correspondence to Dr. R. N. P. Sutton, Department of Medical Microbiology, King's College Hospital Medical School, Denmark Hill, London SE5 8RX.