EB-virus-specific IgM and IgG antibodies in first-degree relatives of children with acute lymphoblastic leukaemia

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Zorbala-Mallios, H., Sutton, R. N. P., and Emond, R. T. D. (1975). Archives of Disease in Childhood, 50, 137. EB-virus-specific IgM and IgG antibodies in first-degree relatives of children with acute lymphoblastic leukaemia. EB-virus-specific IgM and IgG antibodies (to virus capsid and soluble complement fixing antigens) were estimated in sera from mothers and sibs of children with acute lymphoblastic leukaemia, from patients with infectious mononucleosis, and from control individuals. IgM antibodies were present in 12 of 16 mothers and 3 of 4 sibs of children with acute lymphoblastic leukaemia. They were also present in 14 of 16 patients with infectious mononucleosis, but in only 1 of 12 control individuals.

In adult life infection with the Epstein-Barr virus is commonly associated with the development of infectious mononucleosis. During such infections, antibodies to the EB virus-soluble complement fixing antigen develop slowly (Sutton, Marston and Emond, 1971; Vonka et al., 1972). In early childhood, infection with EB virus is usually asymptomatic; when IM occurs the development of EB CF antibodies resembles that in adults (Sutton et al., 1974a). In children with acute lymphoblastic leukaemia, tested early in their illness, the high levels of EB CF antibodies thus imply previous infection with this virus (Sutton et al., 1974b).

In a parallel series of investigations, we observed higher levels of IgM immunoglobulin in mothers of children with ALL than in controls (Sutton, Bishun, and Soothill, 1969). These results have since been confirmed (Chandra, 1972; Hie-Won et al., 1973). Apart from showing infection in the leukaemic children, there were other reasons for suspecting that the EB virus might be associated with this immunological abnormality. The EB virus is associated with malignant disease and with IM; patients with IM have high levels of IgM (Wollheim and Williams, 1966; Sutton et al., 1973), produce autoantibodies, including antibodies to smooth muscle (Holborow, Hemsted, and Mead, 1973; Sutton et al., 1974c) which have also been observed in malignant disease (Whitehouse and Holborow, 1971), and may have temporary impairment of cell-mediated immunity (Haider et al., 1973), of possible significance in oncogenesis (Keast, 1970); following IM there is an increased risk of developing malignant lymphomata and, rarely, IM may be followed by leukaemia (Connelly and Christine, 1974; Levine et al., 1972).

These observations prompted a search for evidence of EB virus infection in relatives of children with ALL. We used a method which differed completely from that used in our earlier work and which was intended to investigate the nature of the raised IgM levels in the mothers. Small volumes of sera and lack of adequate controls prevented us from testing sera from the index cases. Patients with confirmed IM were used as positive controls.

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Abbreviations

ALL: acute lymphoblastic leukaemia
CF: complement fixing
EB: Epstein-Barr (virus)
IM: infectious mononucleosis
VCA: virus capsid antigen

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Materials and methods

The sources of sera from patients with IM, from relatives of leukaemic children, and from controls have been described previously (Sutton et al., 1969, 1971). Sera from 2 apparently healthy mothers of children attending hospital were also included as controls.

All sera were separated within 24-48 hours of collection and stored for varying periods at -70°C or -20°C, without preservative; control and test sera were treated identically.

IgM and IgG fractions were obtained by chromatographic separation on G-200 Sephadex and their identities confirmed by radial immunodiffusion against monospecific antisera. The fractions were pooled by immunoglobulin class and concentrated by ultrafiltration, using an ‘Amicon’ cell, to about 4 times their initial volume. They were then tested by indirect immunofluorescence (Sutton et al., 1973) on P3HR-1 cells for antibodies to EB VCA. P3HR-1 cells contain EB virus and do not produce direct IgM or IgG fluorescence (Nikoskelainen, 1973). Antibodies to EB virus-soluble CF antigen were assayed by methods described previously (Sutton et al., 1971), differing only in that P3HR-1 cells were used as a source of antigen. In these tests the titres obtained related to the approximate initial dilution.

Sera were coded and tested in ignorance of their source. All tests were carried out by the same operator and the code was not broken until all the investigations had been completed. Because of the small numbers of specimens, Fisher’s exact test (Seigel, 1956) was used for determining statistical significance.

Results

Patients with infectious mononucleosis. EB-virus-specific IgM and IgG antibodies were estimated in 18 sera from 16 patients with IM and in 4 control patients with other infectious diseases (Table I). These specimens were comparable with regard to age of patients and length of storage; the patients with IM had a mean age of 19.1 years and their sera had been stored for a mean period of 36.7 months while the control patients had a mean age of 19.3 years and their specimens had been stored for a mean of 31.4 months.

IgM VCA antibody was detected in 14 of 16

<table>
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<th>Case no.</th>
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<th>Weeks from onset of IM</th>
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<th>Months in storage</th>
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<td>F</td>
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</table>

*Reciprocal titres.

TABLE I

EB-virus-specific IgM and IgG antibodies in patients with infectious mononucleosis and in control patients
patients with IM and IgG VCA antibody in 12. IgM CF antibody was not detected in any specimen but IgG CF antibody was present in 15 of 16: titres ranged from 1/4 to 1/256.

Paired sera were available from 2 patients. In one, only IgM VCA antibody was present in the first specimen (1 week after onset), but in the second specimen (5 weeks after onset) both IgG VCA and IgG CF antibodies were found. In the second patient, IgG VCA and CF antibodies were present in the first specimen (2 weeks after onset) and IgM antibody did not appear until 2 weeks later. IgM antibodies were often lacking in sera taken early in the course of disease but were still present in sera taken as long as 88 weeks after onset. Though IgG CF antibodies were found in 2 of 4 control patients, no IgM antibodies were detected in this group.

The prevalence of IgM antibodies was thus greater in patients with IM than in controls (P = 0·0031).

**EB-virus-specific IgM and IgG antibodies**

**First-degree relatives of children with ALL.** EB-virus-specific IgM and IgG antibodies were estimated in 16 mothers and 4 sibs of children with ALL. They were also estimated in 6 healthy mothers of healthy children and in 2 mothers of children attending the same hospital as the patients (Table II). These sera were comparable with regard to age and parity of donor and to length of storage. The mothers of the leukaemic children had a mean age of 33·5 years and a mean of 2·1 children, and their sera had been stored for a mean period of 79·6 months. The control mothers had a mean age of 37·0 years, a mean of 2·45 children, and their sera had been stored for a mean period of 74·9 months. There were no controls for the sibs of the leukaemic children (aged 9 months, 1 year, 9 years, and 15 years).

Twelve of the 16 mothers of children with ALL had EB-virus-specific IgM VCA antibodies; 15 had IgG VCA antibodies. All had detectable IgG CF antibody and none had IgM CF antibody.

**TABLE II**

**EB-virus-specific IgM and IgG antibodies in first-degree relatives of children with acute lymphoblastic leukaemia and in control mothers**

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<th>Case no.</th>
<th>Sex</th>
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<th>EB-virus-specific antibodies to:</th>
<th>Parity</th>
<th>Months in storage</th>
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*Reciprocal titres.

NK, not known.
IgM antibodies were detectable in sera taken up to 220 weeks from the first symptom of leukaemia in their children; more mothers tested long after the onset of leukaemia had IgM antibodies than those tested earlier in the course of their children's disease. None of the healthy control mothers had IgM antibodies; one of the mothers of children attending hospital had such antibodies. All of the mothers of leukaemic children had IgG CF antibodies and 4 of 8 control mothers had these antibodies. 3 of the 4 sibs had IgM VCA antibodies, all had IgG VCA antibodies, and 1 had IgG CF antibodies. The prevalence of IgM antibodies was thus greater in the mothers of children with ALL than in control mothers ($P = 0.0058$).

**Discussion**

The presence of specific IgM antibody implies active antigenic stimulation, either as a result of recent or possibly of persistent infection. The presence of EB-virus-specific IgM antibodies in patients with active IM, where the EB virus is the known cause (Henle, Henle, and Deihl, 1968; Evans, Niedermann, and McCollum, 1968), and not in those with rubella or other infections is therefore not surprising and confirms the reliability of our methods. Their persistence in patients tested long after apparent recovery was not entirely unexpected, for total IgM levels in these patients may remain raised for up to 2 years after apparent recovery (Sutton et al., 1973) and virus may be excreted orally for several months (Miller, Niedermann, and Andrews, 1973; Golden et al., 1973). These raised total IgM levels were not associated with persistence of autoantibodies (such as those to smooth muscle) which are frequently found in patients with IM (Sutton et al., 1974c). Incidentally, EB-virus-specific IgG antibody was present, in one patient, 15 days after onset and IgM antibody developed later; this gives credence to the suggestion of Stevens, Pry, and Manaker (1970) that IM may not always be a primary infection with EB virus.

EB-virus-specific IgM antibodies were present in more mothers of children with ALL than in controls and were also present in 3 of 4 sibs of the leukaemic children, suggesting active antigenic stimulation with the EB virus in these families. These findings may represent an abnormal host response or they may be more directly related to the leukaemic process.

Infection with EB virus, possibly via blood transfusion (Gerber et al., 1969), is well recognized during the course of ALL (Lampkin, Canales, and Mauer, 1967; Ragab and Vietti, 1969; Deardorff, Gerber, and Vogler, 1970; Stevens et al., 1971) and the possibility of maternal infection from their children cannot be discounted. Nevertheless, many children with newly diagnosed leukaemia have evidence of prior infection (Sutton et al., 1974b) and it seems unlikely that the excess of recent infections in the mothers can be entirely coincidental.

Persisting EB-virus-specific IgM antibody and total IgM levels could be due to some congenital immunological abnormality, possibly involving an inability to switch from IgM to IgG synthesis. If this were so, then persistence of IgM antibody to other viral antigens might be expected. An association between hereditary immunodeficiency and leukaemogenesis in mice has been described by Heiniger et al. (1974), who suggested that defective collaboration between different lymphoid cell types and deficiency in proliferation of immunocompetent cells could result in ineffective immunosurveillance against leukaemogenesis. Some such defect may exist in these families and infection with EB virus could easily influence an already critically poised level of immunological competence. The commanding site of replication of EB virus in the reticuloendothelial system would place it an advantage vis-à-vis other infective agents.

There are other possible mechanisms (Sutton et al., 1974b; Zorbala-Mallios and Sutton, 1974) whereby EB virus could promote the onset of leukaemia. Apart from these, we consider that the presence of EB-virus-specific IgM antibody in the mothers of children with ALL could explain the raised levels of total IgM found in these women and is compatible with previous infection with this virus in the index cases. The different approaches and techniques strengthen our conclusion that EB virus infection is associated in some way with ALL; there is reason to suppose that this association may be more than fortuitous.

We are grateful to Dr. Hillas Smith, Coppetts Wood Hospital, to Professors R. M. Hardisty and J. F. Soothill and the consultant staff of The Hospital for Sick Children, Great Ormond Street, London, for help in this investigation, to the individual mothers who kindly provided specimens, and to Dr. J. Nikoskelainen, University of Turku, Finland, for a generous gift of P3HR-1 cells. Financial support was provided by the Medical Research Council.

**References**


EB-virus-specific IgM and IgG antibodies


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