

EB virus-specific IgA in serum of patients with infectious mononucleosis and of healthy people of different ages

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SUMMARY The following sera were tested for EB virus-specific IgA: serial sera from 61 cases of infectious mononucleosis (IM) and from 195 EBV IgG positive healthy students; single sera from each of 1469 persons of different ages, 63 cases of untreated Hodgkin's disease, and 22 neonates.

EBV specific IgA was found in the sera from 88% of cases of IM, from 18.5% of EBV IgG positive healthy students, and in 13.5% of repeat samples from the same students three years later. The incidence of EBV IgA varied from 5 to 30% at different ages in single sera from EBV IgG positive persons aged 2 to 70 years. The higher percentages occurred in the age groups where recent sero-conversion rates were high. Fifteen percent of sera from cases of Hodgkin's disease were positive for EBV IgA, an incidence similar to that for healthy adults in the age group 25-45 years. None of the EBV IgG positive sera from neonates gave a positive reaction for EBV IGA.

On primary infection with EB virus, serum IgG specific for the viral capsid antigen (EBVCA) is produced within a few days. The initial rise occurs usually before any laboratory investigation has been made, and after this the antibody titre varies little and persists for life. We have shown that EB virus specific IgM can be demonstrated in every patient with infectious mononucleosis (IM), that it develops early, and that it disappears within a few months; two-thirds of our sera were negative at three months and all at one year (Blake *et al.*, 1976). Detailed studies of IgA in IM or in a healthy population have not been reported, but Ho *et al.* (1976) did not find IgA in any of their control subjects, and Henle and Henle (1976) found that less than 5% of the controls were positive in their surveys of IgA.

Both these groups of workers found high titres of EBV IgA in serum from over 90% of cases of nasopharyngeal carcinoma. Ho *et al.* (1978) have suggested that the finding of a high serum EBV IgA titre might be an indicator for persons liable to develop nasopharyngeal carcinoma. They reported IgA in the serum of 28% of patients with Burkitt's lymphoma. No studies of serum IgA have been reported in Hodgkin's disease. This disease, however, is of some interest because Rosdahl *et al.* (1974)

and Muñoz *et al.* (1978) reported a slightly higher incidence of Hodgkin's disease in subjects whose sera had been positive for heterophil antibody (HA) within the last 10 years than in HA-negative controls.

For these reasons we have studied: the rise and fall of EBVCA IgA in serial samples from university students developing IM; the presence of EBV IgA in serial samples from healthy students; and the incidence of IgA in single sera from subjects of all ages and from untreated cases of Hodgkin's disease. Our aim was to determine: at what stage EBV IgA appeared after infection; its rate of disappearance, if at all, from the serum; and the expected incidence of sera positive for IgA in the population.

Material and methods

The following sera were examined:

- 1 serial sera, including pre-illness and post-convalescent sera, from 61 cases of IM from the university study partially reported in 1971 (University Health Physicians and Public Health Laboratory Service Laboratories, 1971);
- 2 serial sera from healthy students known to have IgG antibody for EB virus; 140 at university and 55 at a boarding school for boys (Hoskins *et al.*, 1976);
- 3 1469 single sera sent to the Cross-Infection Refer-

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ence Laboratory at Colindale for anti-streptolysin O (ASO) antibody estimation, representing patients of all ages;

- 4 63 sera from untreated cases of Hodgkin's disease, kindly supplied by Professor Peckham and Dr. B. Jameson from the Royal Marsden Hospital;
- 5 22 sera from babies less than 6 weeks old who were found to have maternal EBV IgG.

TESTS

- 1 Sera of all IM cases were screened for IgG, IgA, IgM, and HA; IgG was also titrated.
- 2 All ASO sera from children under 10 years were screened for IgG, IgA, and IgM. Any positive for IgM were also tested for HA.
- 3 For reasons of economy, the remaining ASO sera and the sera from healthy students were screened for IgG and, if positive, for IgA. IgM and HA tests were performed only on those sera positive for IgA in order to identify unrecognised acute IM.
- 4 All sera positive for either IgM or IgA were tested for rheumatoid factor. When enough serum was available IgM tests were repeated on the macroglobulin fraction from a sucrose density gradient.
- 5 Thirty-eight sera found strongly positive for IgA were titrated out.

METHODOLOGY

The indirect immunofluorescence tests for EBVCA IgG have been described by Sumaya *et al.* (1975). For IgG and IgA, an acetone-fixed preparation from a suspension of an EB3 cell line was incubated with a dilution of 1 in 8 of the unknown serum for 1 hour at 37°C and, after washing, was treated with either anti-human IgG/FITC or anti-human IgA/FITC conjugate. IgM was detected as described by Edwards and McSwiggan (1974). A preparation from P3HR1 cell line (sometimes called HR-1K) was incubated with a dilution of 1 in 8 of the unknown serum for 3 hours and, after washing, treated with anti-human IgM/FITC conjugate.

The conjugates for IgG and IgM tests were obtained from Wellcome Research Laboratory and the IgA test from Dako-immunoglobulins. The class-specificity of the anti-human IgA conjugate was confirmed by performing parallel titrations on six sera strongly positive for EBV IgA. The conjugate was used unabsorbed and after absorption with a sample of purified IgA supplied by Dr. Drew of the Department of Experimental Pathology, Birmingham. Negative results were obtained in the tests using the absorbed conjugate. A further check on the specificity of the anti-IgA conjugate was the examination of EBV IgG positive sera from 22 infants under 6 weeks of age. The EBV IgG present in these sera was presumed to be of maternal origin because IgM

was absent. It is known that IgA does not pass the placental barrier so the infant sera should be devoid of IgA. The IgG titres ranged from 1 in 16 to 1 in 512. None of these sera reacted in the IgA test. The absence of IgA staining, even by the four sera with titres of 1 in 512, indicates that the anti-human IgA conjugate is not reacting with IgG subclasses 1, 2, and 4 (Mawhinney *et al.*, 1973).

The presence of HA was detected in the IM sera by the full Lee Davidsohn technique by Dr. McSwiggan at the Central Middlesex Hospital, London (University Health Physicians and Public Health Laboratory Service Laboratories, 1971). The other sera were tested by the Monospot slide test in our laboratory (Ortho Diagnostics Inc). This test uses horse erythrocytes and a short prior absorption with guinea-pig kidney emulsion or ox cell stroma.

Rheumatoid factor was detected at a dilution of 1 in 8 with the latex Rheuma-Wellco Test (Wellcome Research Laboratories).

Results

Figure 1 compares the proportions of the serial sera from 61 cases of IM occurring in the university population which were positive for each class of EBV-specific immunoglobulin and for HA before and at different times after the onset of illness. Sera were positive for IgG in the majority at onset and always thereafter. All sera were positive for IgM and HA in the onset sample and/or the convalescent sample. The HA test remained positive in more patients and for a longer period than the IgM; it was still present in two-thirds of cases at four months and in one case at 13 months after onset. IgA, on the other hand, was detected in only 88% of the acute and/or the convalescent serum. After three months only 20% were still positive, but IgA was still detectable 18 to 30 months later in 10%.

Sera from 140 healthy female university students were selected because the students had had EBV IgG in their serum at the time of entry to the university and no recent history of IM. It was supposed that although a few might have had a recent silent infection the majority would have been infected some months or years previously. The sera taken at entry and at graduation three or four years later were tested for IgA. In no case was IgA found in the later specimen and not in the former; 26 (18%) were positive at entry, and 19 (13.5%) were still positive at graduation.

Similarly, of 55 boys who had serum EBV IgG on entry to boarding school at 11 to 13 years, five also had IgA. Four of these five were positive two years later, and, of the three we were able to test at three and four years, all were still positive.

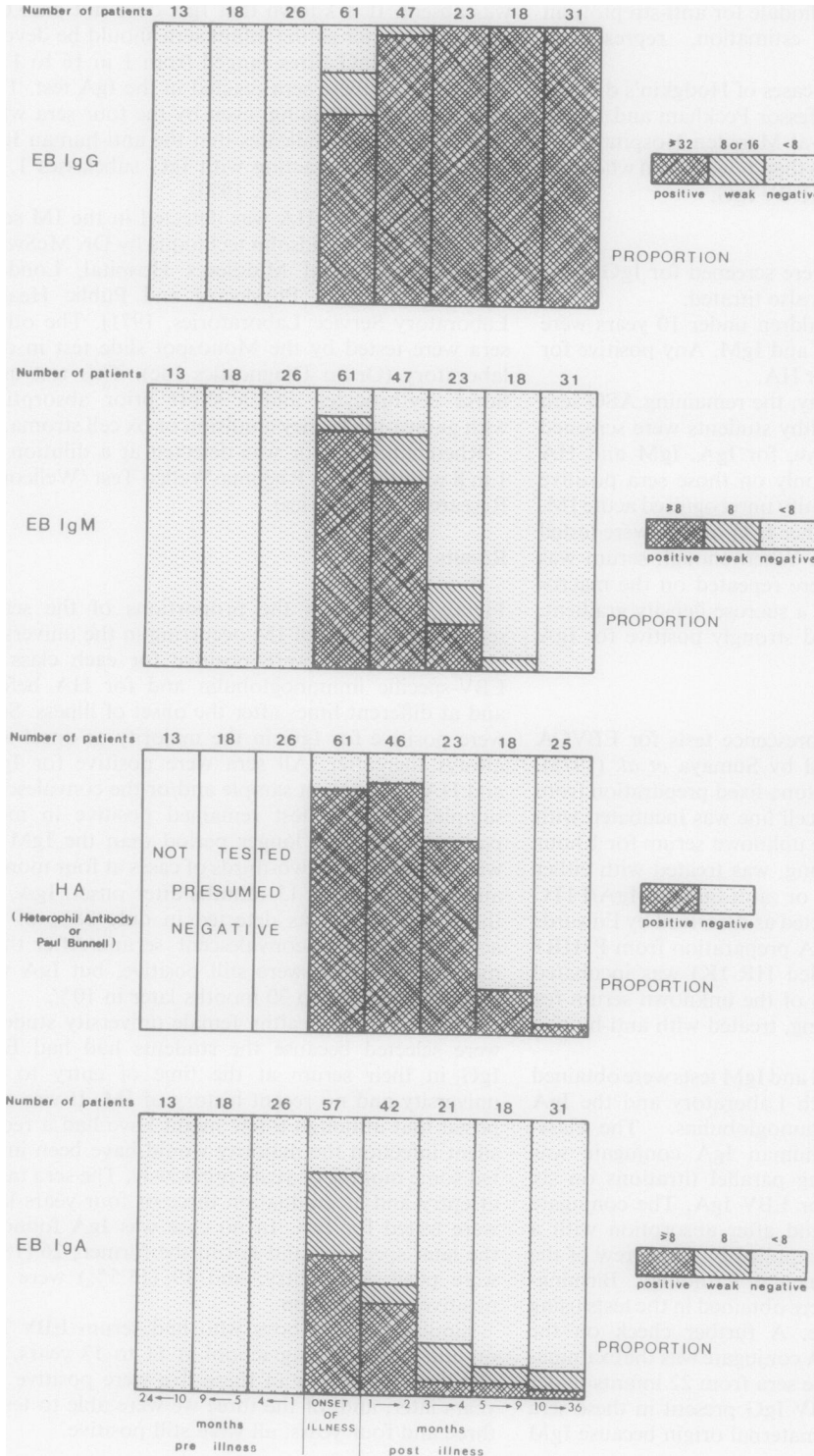


Fig. 1 Illustrates seroconversion and persistence of EB virus specific IgG, IgM, and IgA and heterophil antibody in 61 cases of infectious mononucleosis.

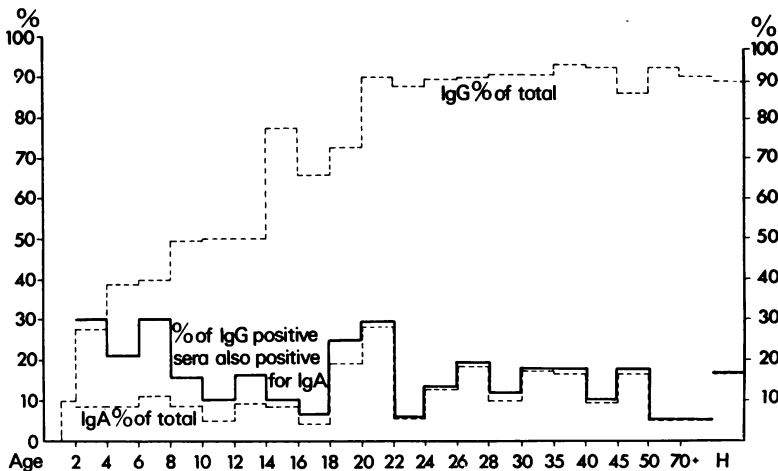


Fig. 2. Shows the percentage of persons whose sera were positive for EBV IgG and EBV IgA (dotted lines) at different ages and the percentage of those positive for IgG who were also positive for EBV IgA (heavy line). The numbers tested in each age group varied from 40 to 200. Larger numbers were tested in the age range 2-12 years and smaller numbers in the ages over 12 years. $H=63$ cases of untreated Hodgkin's disease.

In Fig. 2 the upper and lower lines show the percentage of ASO sera positive for EBV IgG and EBV IgA, respectively, plotted against age. The third, heavy line shows the percentage of the IgG positive sera that are positive for IgA.

The incidence of EB virus infection in relation to age, as shown by the IgG curve, is similar to that found by Pereira *et al.* (1969). There is a higher incidence of infection at two periods, from 1 to 6 years and from 14 to 20 years, resulting in steep rises in the curve from 0 to 40% and from 51 to 90% at these ages. The percentage of sera positive for IgA of the total number at each age is affected in the lower age groups by the small number of EBV-infected persons. The heavy line shows the sera positive for IgA as a percentage of the number positive for IgG. This varies from 5 to 30%, and there are two peaks coincident with or slightly after the steep rises in the IgG line.

The percentage of the Hodgkin sera positive for IgG was the same as that for the adult population (90%), and the percentage of IgG positive Hodgkin sera also positive for IgA was also similar at 16%.

The EBV IgM test was positive on the whole serum in 16 of the ASO sera so the test was repeated on the macroglobulin fraction obtained after centrifugation on a sucrose density gradient. Eight sera were confirmed as IgM positive and presumed to be from cases of acute EB virus infection masquerading as streptococcal infection. Six of these sera were both HA- and IgA-positive. The two HA-negative sera were from children under 3, and it is known that the HA test can be negative in young children (Joncas *et al.*, 1974). Unfortunately, there was insufficient serum to be tested for IgA. The other eight sera were negative after fractionation. Seven of these were positive for rheumatoid factor, which was the prob-

able cause of the false result in the test on whole serum.

TITRATION RESULTS

EBV IgA was usually detectable only at a 1 in 8 dilution, but 38 of the sera showing strong reactions at 1 in 8 were titrated further and only one gave a titre over 1 in 64. In contrast, a case of nasopharyngeal carcinoma gave a serum titre of 1 in 512.

Discussion

There is therefore some suggestion from these results that IgA is produced during the acute illness in quantities nearly always detectable by our methods but that in 80-90% of patients it decreases to a level that we cannot detect within a few months, similar in its time scale with the disappearance of IgM. In contrast with IgM, however, 10-20% of patients maintain a detectable amount of IgA for many years, possibly for a lifetime. It would be interesting if these 10-20% are long-term excretors of virus, such as those described by Miller *et al.* (1973).

The titres of IgA detected in our sera are not as great as the majority of those measured in cases of nasopharyngeal carcinoma and their relatives by Henle and Henle (1976) and Ho *et al.* (1978). It is interesting to note that the concentration of IgA in positive sera in our population lies within such narrow limits. As nasopharyngeal carcinoma associated with EB virus has not been described in the United Kingdom their suggestion that the presence of IgA may be an indication of susceptibility to this disease is not relevant here.

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