Clin. exp. Immunol. (1973) **13**, 359–366.

IMMUNOGLOBULINS AND EB VIRUS ANTIBODIES IN INFECTIOUS MONONUCLEOSIS

R. N. P. SUTTON, KAY REYNOLDS, JENNIFER P. ALMOND, SALLY D. MARSTON AND R. T. D. EMOND

Department of Medical Microbiology, King's College Hospital Medical School, London, and the Royal Free Hospital Infectious Diseases Unit, Coppetts Wood Hospital, London

(*Received* 19 *July* 1972)

SUMMARY

Antibodies to EB virus capsid, EB virus complement-fixing antibodies and IgM, IgA and IgG immunoglobulins were estimated in sera from seventy-four patients with infectious mononucleosis, eighty-nine control patients (mostly with infectious diseases) and 232 healthy medical students and nurses. Complement fixing (but not virus capsid) antibodies were much lower in patients tested during active infectious mononucleosis than in patients tested during late convalescence or in controls, a disparity which could form the basis of a diagnostic test. Immunoglobulin levels were higher in patients with active infectious mononucleosis than in controls and, when tested 6 months to 2 yr later, were still higher than those in healthy individuals.

INTRODUCTION

In a number of infectious diseases, high levels of immunoglobulins (especially IgM) are commonly observed. Infectious mononucleosis is one such condition and its association with EB virus infection is now well recognized (Henle, Henle & Diehl, 1968; Evans, Niedermann & McCollum, 1968). The complete significance of these increases in immunoglobulin is still unclear, and, as part of a continuing study of infection with the EB virus, we have examined sera from patients with infectious mononucleosis and from control groups for antibodies to EB virus capsid and soluble complement-fixing antigens and for IgM, IgA and IgG immunoglobulin content. The results of these investigations and their interpretation are given in the following report.

MATERIALS AND METHODS

Sera were taken from patients admitted to the Royal Free Hospital Infectious Diseases Unit, Coppetts Wood Hospital, London. Infectious mononucleosis was diagnosed in some

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

of these patients, the criteria for diagnosis being clinical, together with a positive serological test and the presence of typical abnormal cells in the peripheral blood. Late in convalescence (160–677 days after the onset of illness), some of these patients were tested again. Patients admitted to the same hospital with other diagnoses (almost all with infectious diseases) formed one control group, another one being formed from healthy medical students and nurses, born in Europe or North America, from whom a sample of blood was obtained at the beginning of their hospital training. Except when specified, the results from both control groups were pooled for analysis. Sera from these three sources were collected concurrently, separated within 24 or 36 hr of collection, and stored, without preservative, at −20°C.

Serological methods

The EB3 cell line was used as a source of soluble complement-fixing antigen. This line had been obtained from Professor Epstein in 1968 and has been subsequently cultured in a medium consisting of Eagle's MEM (78%), foetal calf serum (20%), 200 mM glutamine (1%) and non-essential amino-acids (1%) with added penicillin and streptomycin. The cells were grown in 250-ml flasks, 50 ml of cell suspension to each flask and were incubated at 37°C. An antigen was prepared by adjusting the cell count to 2×10^7 cells/ml and disrupting the cells by four cycles of freezing and thawing, the antigen being then clarified by centrifugation. The micro-method of Tatatsy (1955) as adapted and modified by Sever *et al.* (1964), was used in complement fixation tests. Assay of antibodies to the EB virus capsid antigen was carried out by the method of Henle & Henle (1966), slightly modified in that the microscope slides were treated with a polytetrafluorethylene aerosol to give fourteen clear spaces, each about 3 mm in diameter, in which the EB3 cell smears were spread. Immunoglobulins were estimated by a modification of the single diffusion precipitin method of Mancini, Carbonara & Heremans (1965), results being expressed as a percentage of the Medical Research Council standard serum.

In all types of test, sera were coded and examined in ignorance of their origin and particular techniques were always carried out by the same operator. Test and control sera were examined together in the same experiments in batches of from 16 to 36. As the sex ratios in control and test groups were almost unity and as the numbers were relatively small, the results for both sexes were pooled for analysis. The ages in control and test groups did not differ significantly (the geometric mean of the whole population being 20·6 yr). Logarithmically transformed data were used in the calculation of Student's *t*-tests.

RESULTS

Antibodies to EB virus

Soluble EB virus complement-fixing (CF) antibodies (distributed normally with a median of 28·7) were present in 243 of 321 control individuals. In sixty-six patients with infectious mononucleosis, the distribution was skewed towards the lower levels and only thirteen sera (20%) taken during the acute phase of illness had antibody levels of over 16, a proportion which rose until, in twenty-two patients tested more than 150 days after the onset of illness, seventeen (77%) had antibody levels which were greater than 16 (Table 1).

EB virus capsid antigen (VCA) antibodies (distributed normally with a median of 43·5) were present in 106 of 174 controls. In patients with infectious mononucleosis, the distribu-

tion curve was flattened. VCA antibodies were found in thirty-one of forty-eight sera taken during the first 30 days of illness and, thereafter, the proportion of sera with VCA antibodies rose but did not differ significantly from the control levels.

TABLE 1. VCA and CF antibodies in patients with infectious mononucleosis and in controls

	Patients with infectious mononucleosis tested			
	< 30 days after onset	30–150 days after onset	> 150 days after onset	Controls
VCA antibody				
Eight or greater	31 (65%)	7 (78%)	15 (75%)	106 (61%)
Number tested	48	9	20	174
Significance of difference between patients and controls	$P<0{\cdot}5$[N.S.] (χ^2) test	$P = 0{\cdot}35$[N.S.] (exact test)	$P<0{\cdot}5$[N.S.] (χ^2 test)	
CF antibody				
Sixteen or greater	13 (20%)	2 (17%)	17 (77%)	172 (54%)
Number tested	66	12	22	321
Significance of difference between patients and controls	$P<0{\cdot}0005$ (χ^2 test)	$P = 0{\cdot}02$ (exact test)	$P = <0{\cdot}05$ (χ^2 test)	

TABLE 2. EB virus VCA and CF antibodies in patients with infectious mononucleosis and in controls

		Patients with infectious mononucleosis		Controls
		Tested < 30 days after onset	Tested > 150 days after onset (%)	
Number of individuals		38	20	184
Individuals showing differing EB virus antibody patterns				
V.C.A.	C.F.			
High*	Low†	50%‡	15%§	15%
High	High	13%‡	60%§	47%
Low	High	13%§	15%§	10%
Low	Low	24%§	10%§	28%

* High V.C.A. antibodies = 8 to >128.
† Low C.F. antibodies = <16.
‡ Significant difference from control figures (χ^2 test; $P<0{\cdot}0005$ in both cases).
§ No significant difference from control figures.

Comparison of VCA and CF antibody levels revealed considerable differences between sera taken from patients with acute infectious mononucleosis, controls and patients tested late in convalescence (Table 2). Thus more patients with active illness possessed low levels

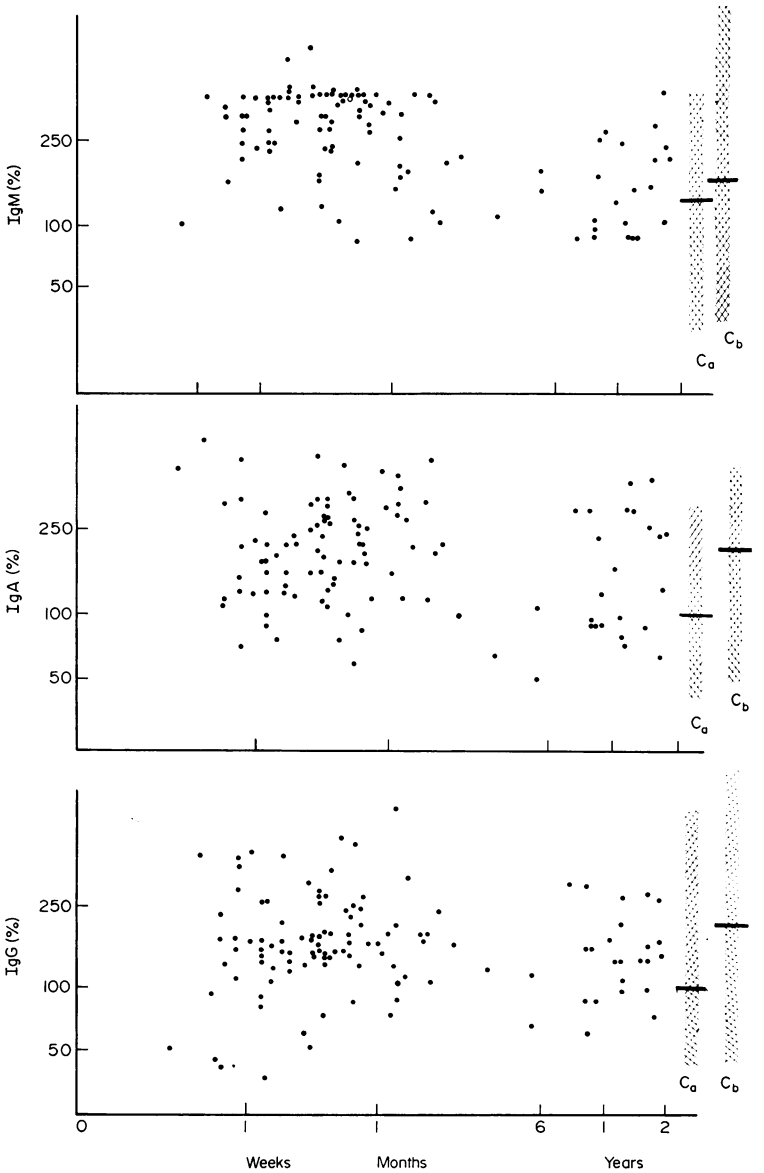

FIG. 1. The decline of immunoglobulins in patients with infectious mononucleosis following the onset of illness. C_A = Range of immunoglobulin levels in control medical students and nurses. Cross bar indicates geometric mean level. C_B = Range of immunoglobulin levels in control patients. Cross bar indicates geometric mean level.

of CF antibody (i.e. sixteen or less) plus VCA antibody than was the case in the other groups. Similarly, a high proportion of patients in control and convalescent groups possessed antibodies of both types, unlike the patients with acute disease.

The antibody pattern observed in acute infectious mononucleosis was also seen, on occasion, in healthy medical students and nurses. Eighteen sera which showed this suggestive pattern were tested for heterophil antibodies by the Monospot test; one showed a reproducible positive reaction. Unfortunately, peripheral blood films were not available from these individuals who were, of course, quite asymptomatic.

Immunoglobulins

IgM, IgA, IgG immunoglobulins in patients with acute infectious mononucleosis were higher than those observed in control groups and these levels declined slowly (Fig. 1),

TABLE 3. Geometric mean values and Student's '*t*'-test analysis for immunoglobulins in patients with infectious mononucleosis control medical students and nurses and control patients

	Immuno-globulin M.R.C. standard serum (%)	Number tested	Student's '*t*'-test analyses			
			Active I.M.	Late I.M.	Healthy control	Control patient
IgM						
Active I.M. (< 30 days after onset)	294·9	69	—	$t = 5{\cdot}45$, $P = <0{\cdot}001$	$t = 17{\cdot}48$, $P = <0{\cdot}001$	$t = 9{\cdot}20$, $P = <0{\cdot}001$
Late I.M. (> 150 days after onset)	140·8	23	—	—	$t = 1{\cdot}88$, $P = <0{\cdot}10$	$t = 5{\cdot}20$, $P = 0{\cdot}001$
Healthy controls	119·2	172	—	—	$P = <0{\cdot}05$ in 1-tailed test	$t = 4{\cdot}15$, $P = <0{\cdot}001$
Control patients	151·6	82	—	—	—	—
IgA						
Active I.M.	204·9	69	—	$t = 1{\cdot}78$, $P = <0{\cdot}1$	$t = 7{\cdot}84$, $P = <0{\cdot}001$	$t = 4{\cdot}56$, $P = <0{\cdot}001$
Late I.M.	155·6	23	—	$P = <0{\cdot}05$ in 1-tailed test.	$t = 3{\cdot}02$, $P = <0{\cdot}005$	$t = 1{\cdot}37$, $P = <0{\cdot}2$
Healthy controls	101·3	169	—	—	—	$t = 0{\cdot}68$, $P = 0{\cdot}9$
Control patients	122·8	82	—	—	—	—
IgG						
Active I.M.	189.9	69	—	$t = 1{\cdot}15$, $P = <0{\cdot}4$	$t = 7{\cdot}89$, $P = <0{\cdot}001$	$t = 1{\cdot}25$, $P = <0{\cdot}3$
Late I.M.	164·5	23	—	—	$t = 3{\cdot}88$, $P = <0{\cdot}001$	$t = 0{\cdot}02$, $P = <0{\cdot}99$
Healthy controls	118·7	171	—	—	—	$t = 4{\cdot}33$, $P = <0{\cdot}001$
Control patients	163·3	82	—	—	—	—

although many sera taken as long as 1 or 2 yr after the onset of illness still had values which were above the mean levels for the controls. As the sera were not taken at

uniform intervals of time, it was not possible to construct reliable regressions of immunoglobulin level upon time which would cover the entire period of illness and convalescence. Nevertheless, comparison of sera taken in the acute phase of illness with those taken late in convalescence (Table 3) shows significantly high levels of IgG and IgA and possibly IgM in these late specimens when these are compared with those from healthy individuals.

The relation of immunoglobulin levels to EB vitus antibodies

Although there was a clear association between infectious mononucleosis and the development of CF antibodies to the EB virus and, similarly, high levels of immunoglobulin were constantly observed during the acute phase of illness, there was no discernible relationship between immunoglobulins (whether IgM, IgA or IgG) and EB virus antibodies (either VCA or CF). In particular, the immunoglobulin levels tended to fall and the virus antibody levels to rise.

DISCUSSION

The relation between EB virus infection and infectious mononucleosis is now well known. During such infections, it appears that VCA antibodies develop rapidly and soluble CF antibodies develop more slowly. The significance of the soluble CF antibodies is not yet clear; thus they are found in cell lines, including those derived from patients with infectious mononucleosis, where there are neither virus capsid antigen detectable by immunofluorescence nor virus particles detectable by electron microscopy (Pope, Horne & Wetters, 1969; Marston *et al.*, 1972). Soluble CF antibodies are found more often in individuals with a past history of infectious mononucleosis than in others and develop regularly during this condition (Sutton, Marston & Emond, 1971). Their tardiness in development, relative to the VCA antibodies, offers a means whereby (using tests for both antibodies) recent asymptomatic infections or infections with unusual clinical manifestations can be distinguished from past infections (Sutton *et al.*, 1971). A certain proportion of the healthy medical students and nurses whom we tested showed a pattern of EB virus antibodies indicative of recent infection and the presence of heterophile antibodies in one of these, who was quite asymptomatic at the time, suggests that he had a silent EB virus infection. As peripheral blood films from these individuals were, unfortunately, not available, we can only speculate upon the possibility of their being cases of subclinical infectious mononucleosis.

Our patients with infectious mononucleosis had high levels of total immunoglobulins (especially the IgM fraction), an observation which was previously made by Wollheim & Williams (1966) who estimated that heterophile antibodies accounted for perhaps 5% of the total IgM immunoglobulin. We also observed high levels of immunoglobulins in patients tested at periods of up to 2 yr after the onset of illness, at a time when they had, apparently, fully recovered. This persistent elevation over such a long period has not, to our knowledge, been recognized before. Three possible causes may be adduced for this unexpected finding. They may be due to chronic virus infection, to the persistence of 'inappropriate antibodies' (Waldenström, 1968) and to variation in host response. The possibility of chronic infection has been suggested by the recent demonstration of IgM antibodies to EB virus during early convalescence (Banatvala, Best & Waller, 1972) and, more directly, by the detection of herpes-like viruses in leucocyte cultures derived from normal individuals

(Moore, Gerner & Franklin, 1967). The EB virus, by virtue of its habitat, is in a favourable position to distort humoral immune responses and to stimulate the production of 'inappropriate antibodies'. Anti-nuclear factor and anti-'i' (Wollheim & Williams, 1966), rheumatoid factor (Dresner & Trembly, 1959; Holborow *et al.*, 1963) and cryoproteins (Kaplan, 1968) have been detected in patients in the acute phase of infectious mononucleosis. We also have made similar observations and these will be described elsewhere. The final possibility, that of host variation in response to infection with the EB virus, cannot be overlooked. Infection with the EB virus, as judged by antibody levels, is widespread throughout the world but some of its commonly accepted manifestations (e.g. Burkitt's lymphoma, nasopharyngeal carcinoma) are almost unknown in temperate zones. Similarly, only a minority of individuals develop clinical infectious mononucleosis, although most are infected at some stage of life with this virus. An acquired immunological difference, resulting from malaria, has been postulated in patients who develop Burkitt's lymphoma (Burkitt, 1969) and there is some experimental confirmation for this (Salaman, Wedderburn & Bruce-Chwatt, 1969). We may speculate that another immunological difference, possibly congenital in this case, separates those who develop infectious mononucleosis from the remainder. One facet of this difference could be an inordinate response in total immunoglobulin production to EB virus infections and also to other infections, such as the frequent respiratory infections which are common to all (Sutton, 1962, 1965) and to which our patients would surely have been exposed during their convalescence. Further investigations will doubtless disclose whether one, or all, of these possibilities accords best with the observed facts.

ACKNOWLEDGMENTS

We are grateful to Dr Hillas Smith and to Dr F. J. Pounds for access to patients and to nurses, and to Dr M. de L. Coutinho for help in collecting specimens. We are especially grateful to Professor J. F. Soothill for help in establishing our immunological techniques. We are grateful to the Medical Committee, King's College Hospital, the Leukaemia Research Fund, and the Cancer Research Campaign for financial support and to Ortho Diagnostics for the provision of reagents.

REFERENCES

BANATVALA, J.E., BEST, J. & WALLER, D.K. (1972) Epstein–Barr virus-specific IgM in infectious mononucleosis, Burkitt's lymphoma and nasopharyngeal carcinoma. *Lancet*, **i**, 1205.

BURKITT, D.P. (1969) Etiology of Burkitt's lymphoma—an alternative to a vectored virus. *J. Nat. Cancer Inst.* **42**, 19.

DRESNER, E. & TREMBLY, P. (1959) The latex fixation reaction in non-rheumatic diseases. *New Engl. J. Med.* **261**, 981.

EVANS, A.S., NIEDERMANN, J.C. & MCCOLLUM, R.W. (1968) Sero-epidemiologic study of infectious mononucleosis with the EB virus. *New Engl. J. Med.* **279**, 1121.

HENLE, G. & HENLE, W. (1966) Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bacteriology*, **91**, 1248.

HENLE, G., HENLE, W. & DIEHL, V. (1968) Relation of Burkitt's tumour associated herpes-type virus to infectious mononucleosis. *Proc. nat. Acad. Sci. (Wash.)*, **59**, 94.

HOLBOROW, E.J., ASHERSON, G.L., JOHNSON, G.D., BARNES, R.D.S. & CARMICHAEL, D.J. (1963) Antinuclear factor and other antibodies in blood and liver diseases. *Brit. med. J.* **i**, 656.

KAPLAN, M.E. (1968) Cryoglobulinaemia in infectious mononucleosis; quantitation and characterization of the cryoproteins. *J. Lab. clin. Med.* **71,** 754.

MANCINI, G., CARBONARA, A.O. & HEREMANS, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2,** 235.

MARSTON, S.D., ALMOND, E.J.P., BISHUN, N.P., MAUNSELL, E.D. & SUTTON, R.N.P. (1972) A 'virus-free' cell line derived from a patient with infectious mononucleosis. *J. clin. Path.* **25,** 701.

POPE, J.H., HORNE, M.K. & WETTERS, E.J. (1969) Significance of a complement fixing antigen associated with herpes-like virus and detected in the RAJI cell line. *Nature (Lond.)*, **222,** 186.

SALAMAN, M.H., WEDDERBURN, N. & BRUCE-CHWATT, L.J. (1969) The immunodepressive effect of a murine plasmodium and its interaction with murine oncogenic viruses. *J. Gen. Microbiol.* **59,** 383.

SEVER, J.L., LEY, A.C., WOLMAN, F., CAPLAN, B.M., PERNELL, W., CROCKETT, B.S. & TURNER, H.C. (1964) Utilization of disposable plastic plates, with a serologic microtechnic. *Amer. J. clin. Path.* **41,** 167.

SUTTON, R.N.P. (1962) Respiratory viruses in a residential nursery. *J. Hyg. Camb.* **60,** 51.

SUTTON, R.N.P. (1965) Minor illness in Trinidad: a longitudinal study. *Trans. roy. Soc. trop. Med. Hyg.* **59,** 212.

SUTTON, R.N.P., MARSTON, S.D. & EMOND, R.T.D. (1971) Some observations on complement fixing antibodies to the EB virus. *J. clin. Path.* **24,** 801.

SUTTON, R.N.P., ALMOND, E.J.P., MARSTON, S.D. & EMOND, R.T.D. (1971) Complement-fixing and fluorescent antibodies in diagnosis of E.B.-virus infections. *Lancet* **ii,** 1095.

TAKATSY, G. (1955) The use of spiral loops in serological and virological micro-methods. *Acta microbiol. Acad. Sci. hung.* **3,** 191.

WALDENSTRÖM, J.G. (1968) *Monoclonal and Polyclonal Hypergammaglobulinaemia.* Cambridge University Press, 1968.

WOLLHEIM, F.A. & WILLIAMS, R.C. (1966) Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M (IgM). Increase in infectious mononucleosis. *New Engl. J. Med.* **274,** 61.