Some observations on complement-fixing antibodies to the EB virus

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SYNOPSIS Complement-fixing antibodies against an antigen prepared from the EB3 line of cultured Burkitt tumour cells were studied in various groups of patients and control individuals. Higher antibody titres were observed in patients with Burkitt's tumour than in African patients with other diagnoses. Significantly more medical students and nurses with a history of infectious mononucleosis possessed antibodies than those with no such history. Low levels of antibody were observed in patients during the acute phase of infectious mononucleosis and these levels were significantly lower than those in patients admitted to the same hospital with other diagnoses.

During the early months following the acute phase of illness, EB complement-fixing antibodies remained stationary or apparently declined in titre but, in patients tested one or more years later, significantly higher antibody levels were observed.

Since Epstein, Achong, and Barr (1964) demonstrated the presence of a herpes-like virus (the EB virus) in cultured Burkitt tumour cells, many workers have investigated the significance of antibodies to this virus. Thus antibodies have been found in sera from patients with Burkitt tumours and in apparently normal individuals (Henle and Henle, 1966) and, more recently, an association has been recognized between the development of antibodies to the EB virus and infection with infectious mononucleosis (Niederman, McCollum, Henle, and Henle, 1968). Immunofluorescent techniques have been used in almost all these studies and complement-fixing antibodies, although described (Armstrong, Henle, and Henle, 1966; Henle and Henle, 1966; Gerber and Birch, 1967), have not been employed to any great extent. In the following report, we outline our initial experience with a complement-fixing antigen prepared from cultured Burkitt tumour cells and suggest that this may provide an additional tool in the investigation of EB virus infections.

Methods

Sera were obtained from healthy medical students and nurses at the beginning of their hospital training, who also completed a questionnaire in which they were asked whether they had ever had infectious mononucleosis or glandular fever. In the present

study, we only include those born in Europe or North America as preliminary observations suggested considerable differences in antibody levels and in history of illness between those born in temperate climates and those born in the tropics.

Sera were also taken from patients admitted to the Royal Free Hospital Infectious Diseases Unit, Coppelts Wood Hospital, London. Infectious mononucleosis was diagnosed in some 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. Patients admitted to the same hospital with other diagnoses formed a control group. Sera from these patients were tested blindly and the diagnosis was not disclosed until the serological result had been reported. Sera were also obtained from patients admitted to hospitals of the King's College Hospital group. Sera from patients with Burkitt's tumour and from African control patients, matched as far as possible for age and sex, were obtained from the University Hospitals at Makerere and Ibadan. All sera were stored without preservative at -20°C .

SEROLOGICAL METHODS

The EB3 cell line was used as a source of antigen. This line had been obtained from Professor Epstein in 1968 and was subsequently cultured in Eagle's MEM (78%), foetal calf serum (20%), 200 mM glutamine (1%), and non-essential amino-acids (1%) with added antibiotics. The cells were grown in 250

ml flasks, 100 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. In some early experiments, the cells were 'aged' (Old, Boyse, Oettgen, de Harven, Geering, Williamson, and Clifford, 1966) by holding them at 30°C for some days before antigen preparation but, as this gave no apparent advantage in antigen yield, in the majority of cases the cells were incubated throughout at 37°C. The micro-method of Takatsy (1953) as adapted and modified by Sever, Ley, Wolfman, Caplan, Pernell, Crockett, and Turner (1964) was used in complement-fixation tests. Sera were tested in batches of 16 to 24.

IMMUNOFLUORESCENCE

Assay of fluorescent antibodies was carried out by the method of Henle and Henle (1966). The EB3 cell line was used as a source of cells. Sera were coded and randomised before being examined.

Results

Sera from 14 patients with Burkitt's tumour and

from nine African patients with other conditions were tested for antibodies to the EB virus by immunofluorescence and by complement fixation (CF). In both types of assay, antibody titres were, in general, higher in the patients with Burkitt's tumour than in the remainder, although antibodies were present in both groups (Table I).

Two hundred and nineteen sera from healthy medical students and nurses and from patients admitted to hospital with a variety of diagnoses (other than Burkitt's tumour or infectious mononucleosis) were tested for EB complement-fixation antibodies. A steady rise in the proportion of individuals with positive sera, ie, with titres greater than 4 was observed during the years of early adult life (Table II). One hundred and fifty-three of 219 adults (70%) possessed EB complement-fixing antibodies.

Of the 181 nurses and medical students who were included in this study, 18 (10%) gave a history of previous infectious mononucleosis. Such a history appeared to be significantly associated (Table III) with the possession of EB complement-fixation antibodies. However, 46 patients admitted to hospital with infectious mononucleosis tended to have lower EB complement-fixation antibody levels

Age (years)	Sex	Source	Diagnosis	Fluorescent Antibody Titre	Complement-fixing Antibody Titre
4	M	Makerere	Burkitt's tumour	> 640	12
5	M	Makerere	Burkitt's tumour	120	16
5	M	Makerere	Burkitt's tumour	> 640	> 4
23	F	Makerere	Burkitt's tumour	> 640	192
12	M	Makerere	Burkitt's tumour	> 640	256
12	NK	Makerere	Burkitt's tumour	120	8
7	NK	Makerere	Burkitt's tumour	120	8
7 mth	NK	Ibadan	Burkitt's tumour	10	32
8	NK	Ibadan	Burkitt's tumour	120	8
7	M	Ibadan	Burkitt's tumour	> 640	16
5	M	Ibadan	Burkitt's tumour	120	NT
7	F	Ibadan	Burkitt's tumour	60	256
3	F	Ibadan	Burkitt's tumour	120	192
NK	NK	Makerere	Burkitt's tumour	> 640	NT
5	M	Makerere	Intussusception	60	6
6	M	Makerere	Tubercular lymphadenitis	15	8
9	M	Makerere	Hepatic cirrhosis	240	12
30	M	Makerere	Fracture of tibia	120	24
15	M	Makerere	Anaplastic tumour	15	16
5	F	Ibadan	Gastroenteritis	240	20
14	F	Ibadan	Nephrotic syndrome	24	NT
3	M	Ibadan	Tuberculosis	240	> 4
9	F	Ibadan	Nephrotic syndrome	30	> 4

Table I Antibodies to EB virus in African patients

Antibody Levels	
Fluorescent	Complement-fixing
Rank sum (control patients) = 77.2 for $n_1 = 9$; $n_2 = 14$, $R = 81$ $\alpha = 0.05$ (significant at 5% level)	Rank sum (control patients) = 65.0 for $n_1 = 9$; $n_2 = 11$, $R = 68$ $\alpha = 0.025$ (significant at 2.5% level)

Table Ia Significance of difference between antibody levels in patients with Burkitt's tumour and African patients using one-tailed Wilcoxon ranking test

Age (years)	Source	Number with EB Complement-fixing Antibody Titres greater than 4/Total Number Tested	Percentage of Sera with EB Complement-fixing Antibody Titres of 4 or less
18-19	Nurses	35/64	54
20-21	Nurses and medical students	50/82	61
22-23	Nurses and medical students	18/27	67
24-28	Nurses and medical students	6/8	75
Over 25	Hospital patients (other than those with infectious mononucleosis)	34/38	89
Over 18	Total	153/219	70

Table II *EB Complement-fixing antibodies in different age groups*

	EB Complement-fixation Antibody Titres	
	4 or Less	Greater than 4
History of infectious mononucleosis	2	16
No history of infectious mononucleosis	68	95

Table III *EB complement-fixation antibodies in nurses and medical students related to their histories of previous infectious mononucleosis*¹¹P = 0.007 (Fisher's exact test) (significant at 1% level)

	EB Complement-fixation Antibody Titres	
	4 or Less	Greater than 4
Patients with infectious mononucleosis	19	27
Patients with other diagnoses	5	45

Table IV *EB complement-fixation antibodies in patients admitted to Coppetts Wood Hospital*¹¹P = 0.0003 (Fisher's exact test) (significant at 0.1% level)

than 50 patients admitted to the same hospital with other conditions (Table IV). The sex ratio in the two groups of hospital patients was the same but the control patients were older (mean age 30 years) than those with infectious mononucleosis (mean age 18 years). Comparison by age groups suggested that the significance of this difference was not considerably

affected by this inhomogeneity; thus, in the 11-20-year-old age group, 15 of 31 patients with infectious mononucleosis (48%) and two of 12 control patients (17%) lacked EB complement-fixation antibodies.

We followed up 23 patients who had been admitted to hospital, eight to 22 months previously, with infectious mononucleosis and who had remained well since their discharge from hospital. Significant increases in EB complement-fixation antibody levels were observed in almost all of these patients (Table V).

	EB Complement-fixation Antibody Titres	
	4 or Less	Greater than 4
Sera taken during acute phase of infectious mononucleosis (mean 0.4 months after onset)	11	12
Sera taken following recovery from infectious mononucleosis (mean 14.4 months after onset)	1	22

Table V *EB complement fixation antibodies in 23 patients with infectious mononucleosis*¹¹P = 0.0008 (Fisher's exact test significant at 0.1% level)

The EB complement-fixation antibody levels in medical students, nurses, and patients were analysed according to the time which had elapsed between the onset of infectious mononucleosis and the dates on which their sera were taken (Table VI). They were

	Period Elapsed between Onset of Infectious Mononucleosis and Date of Serum		
	15 Days or Less	15 Days to One Year	More than One Year
Number of patients	31	35	38
Geometric means of EB CF titres	44.45	31.43	69.05
Comparison between groups (Student's <i>t</i> test)			
15 days or less	—	<i>t</i> = 0.90 0.40 > <i>P</i> < 0.30	<i>t</i> = 1.43 0.1 > <i>P</i> < 0.2
15 days to 1 year	—	—	<i>t</i> = 2.46 0.01 > <i>P</i> < 0.02

Table VI *EB Complement-fixation antibodies in patients tested at varying times after the onset of infectious mononucleosis*

arranged in three approximately equal groups for statistical reasons. The geometric mean titres of sera taken during the first year remained stationary or possibly declined but the mean titre of sera taken one year or more following illness had risen significantly.

Discussion

It has been shown convincingly (zur Hansen, Henle, Hummeler, Diehl, and Henle, 1967; Epstein and Achong, 1968) that those cells which react in the Henle and Henle (1966) type of fluorescent antibody test contain EB virus. The role of the EB virus in the complement-fixation test is not so clear and complement fixation with antigens derived from apparently virus-free cell lines has been reported (Pope, Horne, and Wetters, 1969). The essential nature of our antigen remains, therefore, an enigma. Nevertheless, we have shown that there is a relationship between development of antibodies to this antigen and a past history of infectious mononucleosis and that those patients who develop infectious mononucleosis are those without preexisting antibody. Although, in this condition, it is often difficult to define accurately the date of onset, we have evidence that the initially modest levels of EB complement-fixation antibody remain stationary or possibly decline during the early months following illness, to be followed, over one year later, by a statistically significant rise in geometric mean titre. These observations are consonant with the hypothesis that episodes of clinical infectious mononucleosis are primary infections with the EB virus and that subsequent re-infection, or re-activation of a latent infection, may take place, possibly in the absence of frank illness.

The variations in EB complement-fixation antibody titres which we have described have not, so far, been seen in cases studied with immunofluorescent or other tests. Further work is clearly necessary to delineate the character of the EB complement-fixing antigen, its relationship to similar antigens from other cell lines, and to immunofluorescent and other antibodies.

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