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Epidemiological studies of Epstein–Barr herpesvirus infection in Western Australia

By P. K. LAI, E. M. MACKAY-SCOLLAY
Division of Microbiology, State Health Laboratory Services, Western Australia
AND M. P. ALPERS*
Department of Microbiology, University of Western Australia, Perth Medical Centre, Shenton Park 6008, *Western Australia*

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SUMMARY

In a study on a Caucasian population in Western Australia the prevalence of antibodies to Epstein–Barr virus (EBV) was 41% in the 9- to 10-year age group, 80% in the 16 to 19-year age group and 92% in young adults. The age-specific annual seroconversion rates indicated two peaks of primary EBV infection in the population studied – one under 5 years of age and the other at adolescence. The geometric mean titre rose with age, from 23 at 5-6 years to 53 at 36–40 years.

It was shown that in 73 families studied there was evidence of probable spread of EBV infection among siblings, particularly between those of the same sex.

Serological study of patients with infectious mononucleosis indicated that 100% of those examined had antibody to EBV and the geometric mean titre was elevated to 210. Rising titres and seroconversion was demonstrated in these patients together with successful establishment of EBV-carrying cell lines from the peripheral blood in two-thirds of the cases.

INTRODUCTION

The Epstein–Barr herpesvirus (EBV), detected in cultured Burkitt lymphoma cells (Epstein, Achong & Barr, 1964), can be found in lymphoblastoid cell lines established from normal subjects who have antibody to the virus, and from patients with various clinical illnesses, including infectious mononucleosis. Repeated attempts to grow this virus in the laboratory with standard techniques have failed, but infection of human haematopoietic cells has been successful, giving rise to transformed cells which proliferate indefinitely *in vitro* (Lai *et al.* 1973*b*).

Serological studies have shown that EBV is broadly disseminated in all human populations studied (Hinuma, Ohta-Hatano, Suto & Numazaki, 1969; Porter, Wimberley & Benyesh-Melnick, 1969; Niederman, Evans, Subrahmanyan & McCollum, 1970) and that patients with Burkitt lymphoma, nasopharyngeal carcinoma or infectious mononucleosis have higher serum antibody titres to EBV

* Senior Research Fellow of the National Health and Medical Research Council of Australia.

than the general population. In a prospective study, Evans & Niederman (1972) reported that 5·5% of EBV-seronegative subjects developed clinical infectious mononucleosis and became seropositive within 1 year. In addition, EBV has been recovered from the oropharynx of patients with infectious mononucleosis (Miller, Niederman & Andrews, 1973).

Infection of non-human primates with EBV and EBV-carrying cells has given rise not only to seroconversion but also to infectious mononucleosis-like symptoms (Werner *et al.* 1972) and Paul-Bunnell heterophil antibodies (Shope & Miller, 1973).

This study extends the observations already published to a Caucasian affluent population living mainly in a country town in Western Australia. EBV antibody prevalence and seroconversion rates have been calculated by age and sex and the pattern of antibodies examined in 73 families. In addition, 58 patients with infectious mononucleosis were investigated.

MATERIALS AND METHODS

Serum samples and patients

Serum specimens collected between 1969 and 1972 from various surveys, especially those from the Busselton Health Survey (Curnow *et al.* 1969; Leading article, 1974), afforded us an opportunity to determine the prevalence of antibodies to EBV in Western Australia. Busselton is a small town in a thriving agricultural district, situated on the coast, 150 miles south of Perth, the capital of Western Australia. It is a stable community with a population of 7000, of which 85% were born in Australia. Serum samples collected from over 95% of the townspeople were stored at $-20°$ C. and one-seventh of these were selected randomly by computer for EBV-antibody titration. Blood samples were also collected, from 1970 to 1973, from patients with clinically diagnosed and haematologically confirmed infectious mononucleosis for the determination of EBV-antibody status and for the establishment of EBV-carrying cell lines.

Immunofluorescence

EBV antibody was measured by the indirect immunofluorescence test (Henle & Henle, 1966) using QIMR-WIL cells (Pope, 1968) as the source of antigen. The cells were air-dried on cover-slips, fixed in acetone and tested for antibody after overlay with dilutions of test sera and staining with fluorescein-conjugated anti-human globulin (Baltimore Biological Laboratories, U.S.A.). Raji cells, which do not exhibit the viral capsid antigen, were treated in the same way and used as controls.

Sera were usually screened at 1/8 and 1/10 dilutions, and independent readings were made by at least two observers, in a majority of the samples, for specific immunofluorescence under ultraviolet illumination with a Leitz microscope, employing a 1 mm. UG 1 excitation filter and a K-450 barrier filter. A single standard human serum was titrated for each new antigen preparation with the requirement of a not more than twofold variation before use. The same positive serum and a

standard negative serum were included on every slide of five serum samples. Saline and conjugate cell controls were checked on each day's test. All test sera were titrated and read under code.

Establishment of cell lines

Heparinized peripheral blood samples collected from patients with infectious mononucleosis were allowed to stand at 37° C. for 1 hr. The leucocyte-rich plasma was collected and centrifuged at 1200 rev./min. for 10 min. The cell pellet was washed twice in Hanks's balanced salt solution and cultured according to the method of Pope (1967) in Roswell Park Memorial Institute medium-1640 (Grand Island Biological Co., N.Y.) supplemented with 20% fetal bovine serum (BioCult, U.K.), 100 units/ml. penicillin and 100 μg./ml. streptomycin. The cell-free plasma was stored at $-20°$ C.

Estimation of percentage of fluorescing cells

The percentage of fluorescing cells in established cell lines was obtained by the method of Lai, Mackay-Scollay & Alpers (1973*a*).

Electron microscopy

Identification of EBV was carried out in two of the established cell lines by electron microscopy according to the method of Lai *et al.* (1973*b*).

RESULTS

Prevalence of EBV antibody

Table 1 shows the age distribution of EBV antibody in the serum samples tested. Antibody to EBV was present in 38% of the children by the age of 6 with a geometric mean titre of 23·2. By the age of 12, 49% of the children tested had antibody to EBV; this rose to 80% by the age of 19 and 92% by the age of 25. The figure continued to rise slowly with age and was 97% in persons over 50 years of age.

Sex and age distribution of antibody

Table 2 shows the sex and age distribution of antibody to EBV. By the age of 10, 42% of the males and 37% of the females in the study had antibody to the virus. This rose steadily to 73% in males and 88% in females by the age of 19. By the age of 25 the prevalence of EBV antibody was 96% in males and 89% in females.

Seroconversion rate

The average annual seroconversion rates over intervening age periods were obtained from the difference in prevalence at the beginning and end of each period. on the assumption that the experience of each cohort with respect to EBV infection has been the same; the rates are plotted at the mid-point of these periods

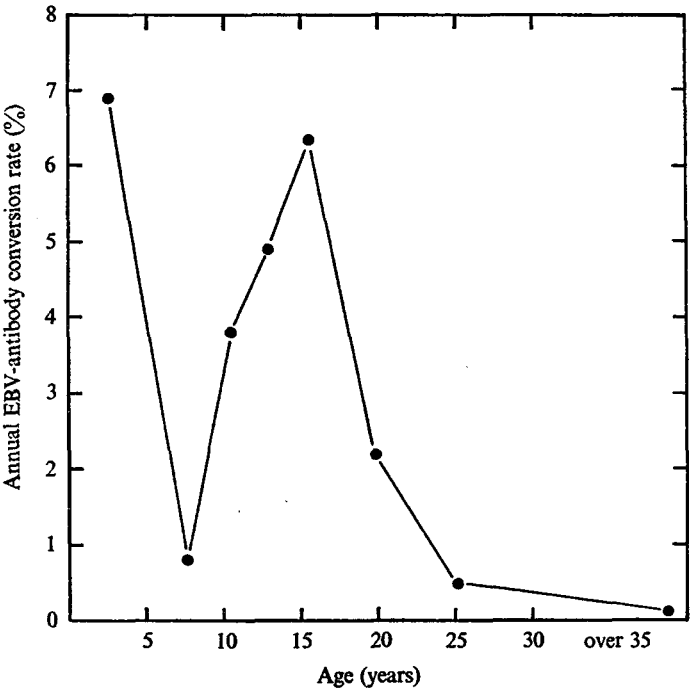

Fig. 1. Age-specific annual EBV-antibody conversion rate. The average annual antibody conversion rate was calculated from the differences in prevalence ratios (number of subjects positive for EBV antibody per 100 subjects tested, as in Table 1) between different ages, divided by the difference in age, and plotted at the mid-point of the interval, for each age interval for which we had data (Table 1). The rate is expressed as a percentage—that is, number of subjects undergoing seroconversion per annum per 100 subjects tested. This calculation assumes that the experience of each cohort with respect to EBV infection has been the same.

in Fig. 1. The highest incidence of EBV infection occurred in the years under 5 and during adolescence.

Prevalence of EBV antibody in families

In 73 families studied in the Busselton population (Curnow *et al.* 1969) the number of siblings ranged from 2 to 4. The intervening years between siblings ranged from 1 to 6 and the age of all the children ranged between ages 6 and 17 years. The sex structure of these families is shown in Table 3.

Both parents in each of the 73 families were found to have antibody to EBV with geometric mean titre of 42·5. The geometric mean titre for the children, calculated from those with EBV antibody present, was 35·4.

In the 73 families studied, no child had detectable EBV antibodies in 17 (23 %) (Table 3). The siblings were of the same sex in 5 of the 17 (29 %). Twenty families (27 %) had EBV antibody in all members of the family; the siblings were of the same sex in 15 of these 20 (75 %). The discordant families, in which one or more but not all the siblings had antibody to EBV, numbered 36 (49 %). There was no significant difference in the geometric mean titre or age range of the siblings between any of the groups shown in Table 3.

Based on data from Table 1 and the age of the siblings, in each of the 73 families studied the probability was estimated of positive concordance in the sibship if infection of each sib by EBV was independent of the nuclear family. The harmonic mean of these 73 probabilities was 16·8%. Similarly, the average probability of negative concordance in a family was estimated as 27·4% and of discordance by the remainder of 55·8%.

Studies on infectious mononucleosis

Single serum samples from 58 patients with infectious mononucleosis were examined. All had antibody to EBV. The geometric mean titre was 209·9. In addition, 12 patients with clinically diagnosed infectious mononucleosis and positive for Paul-Bunnell heterophil antibodies were selected for more detailed studies, the results of which are shown in Table 4.

The mean titre for the 12 cases using the highest titre reached in each case was 183·7. Attempts were made in each case to establish EBV-carrying cell lines. About half of the attempts (13/27) were successful. EBV-carrying cell lines were established from two-thirds (8/12) of the cases.

All established cell lines, when examined for EBV by indirect immunofluorescence 3 months after establishment, were shown to carry EBV. In addition, EBV was identified in two of the cultures examined by electron microscopy. The percentage of EBV-carrying cells was generally low, ranging from 1·8% to 5·6% Moreover, this proportion decreased after 6 months of culture and remained thereafter consistently in the range of $\leqslant 0{\cdot}5$ to 2·4% fluorescing cells.

DISCUSSION

The prevalence of antibody to EBV varies in different populations studied. Henle & Henle (1967) reported a prevalence of EBV antibody of about 50% which persisted in American children of low socio-economic status from the age of 2–4 years through adolescence, and a prevalence of about 80% in adults. In another series Porter *et al.* (1969) observed that the prevalence was 80% in children aged 2–4 years but this rose to 90% by the age of 14. The frequency of EBV-positive sera in 3-year-old children was reported as 80% in a Japanese population by Hinuma *et al.* (1969) and 88·8% in a Huixquilucan population by Golubjatnikov *et al.* (1973), who also found a frequency of 93·5% in Mexican children and 39·4% in American children (Wisconsin) aged between 5 and 9 years. In the present study, it was found that the frequency of EBV-positive sera was 38% in West Australian children (Caucasian) aged 5–6 years (Table 1). The prevalence rose to 41% in the 9–10 year age group, to 80% at the age of 16–19 years and 92% in young adults. Thereafter, this level was maintained throughout life (Table 1).

Seroconversion rates (Fig. 1) indicate two clear peaks of primary EBV infection: one under the age of 5 years and the other at adolescence.

Primary EBV infection in children under 10 years appeared to be more prominent in males (42%) than in females (37%) (Table 2). However, by the age of 19 the frequency of EBV-positive sera was greater in females (88%) than in males

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Table 1. *Prevalence and geometric mean titre of EBV antibody at different ages*

Age groups	No. tested	No. positive	% positive	GM titre*
5–6	55	21	38	23·2
7–8	127	48	38	25·6
9–10	109	45	41	29·8
11–12	106	52	49	31·2
13–15	92	56	61	34·0
16–19	156	125	80	37·2
20–25	142	131	92	42·8
26–30	121	115	95	41·3
31–35	122	114	93	43·6
36–40	123	117	95	52·7
> 40	402	389	97	49·7

*Geometric mean titre calculated from positive titres only. Titre is expressed as reciprocal of the highest dilution of serum which gives fluorescence of EBV-positive cells in the Henle test (Henle & Henle, 1966).

Table 2. *Sex and age distribution of EBV antibody*

Age groups		No. tested	No. positive	% positive
5–10	Male	146	61	42
	Female	145	53	37
11–15	Male	98	51	52
	Female	100	57	57
16–19	Male	76	55	72
	Female	80	70	88
20–25	Male	69	66	96
	Female	73	65	89
26–30	Male	55	54	98
	Female	66	61	92
> 30	Male	330	321	97
	Female	317	299	94

Table 3. *Sex structure of sibship in families studied**

Sex structure	Discordant families†	Positively concordant families‡	Negatively concordant families§	Total
Same sex				
Male	8	10	2	20
	14	15	5	34
Female	6	5	3	14
Opposite sex	22	5	12	39
Total	36 (49 %)	20 (27 %)	17 (23 %)	73

* All parents had antibodies to EBV.
† Discordant families have some sibs positive, some negative for EBV antibody.
‡ Positively concordant families have all sibs positive for EBV antibody.
§ Negatively concordant families have all sibs negative for EBV antibody.

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Table 4. *EBV antibody and establishment of EBV-carrying cell lines from patients with infectious mononucleosis*

Case no.	Interval after 1st blood collection (days)	EBV-antibody Titre*	EBV-carrying cell lines†	Latent period‡ (days)
1	—	20	—	—
	11	40	—	—
2	—	⩽10	—	—
	9	40	—	—
3	—	20	—	—
	18	160	SH—IM1	24
4	—	40	—	—
	16	80	—	—
5	—	80	SH—IM2	32
	15	80	—	—
	101	80	—	—
6	—	40	SH—IM3	31
	8	80	SH—IM4	29
	14	160	SH—IM5	34
7	—	320	SH—IM6	27
	10	320	SH—IM7	30
	21	320	—	—
8	—	10	—	—
	3	10	—	—
9	—	80	SH—IM8	26
	13	80	Not done	
	24	80	SH—IM9	29
10	—	320	SH—IM10	34
	16	320	SH—IM11	32
11	—	80	SH—IM12	25
	14	160	—	—
12	—	80	SH—IM13	24
	14	40	—	—

* EBV antibody detected by indirect immunofluorescence (Henle & Henle, 1966) and expressed as the reciprocal of the highest serum dilution to cause fluorescence of EBV-positive cells.

† EBV-carrying cell lines established according to the method of Pope (1967) in Roswell Park Memorial Institute medium-1640 with 20% fetal bovine serum.

‡ Period between initiation and establishment of culture.

(73%), indicating that the major primary infection in females occurred between 11 and 19 years of age. Thereafter, in males the antibody prevalence rose steeply to reach the adult plateau at 25 years.

These findings are consistent with the epidemiology of infectious monunucleosis in Western Australia (Hubble, Lai, Mackay-Scollay & Alpers, 1974), where a minor peak of clinical infectious mononucleosis was detected in children aged under 10, with males predominant, and the major peak in late adolescence, in females at 15–19 years and in males at 20–25.

Parental transmission of EBV throughout a nuclear family appeared to be an unusual event. Of the 73 families studied, all parents had EBV antibody, but only

in 20 families was antibody to EBV detected in all the siblings of the family. Henle & Henle (1970) suggested that the source of early childhood EBV infection might be parental but that transmission would not be a regular event.

In this study, the observed proportions of positive and negative concordance and discordance for EBV antibodies, in the siblings of the 73 families studied, were 27%, 23% and 49% respectively (Table 3). The proportions estimated on the assumption that EBV infection in each sibling was an independent event and taking age and sex into consideration were 17%, 27% and 56% respectively. The difference between the observed and the estimated positive concordance was highly significant statistically ($P < 0{\cdot}001$), indicating transmission of EBV among siblings of the same family. In addition, it became evident from Table 3 that such transmission of EBV horizontally in a nuclear family was more likely to have occurred among siblings of the same sex than those of opposite sex ($\chi^2 = 5{\cdot}96$, D.F. = 1, $P < 0{\cdot}02$). In the age range of siblings studied, 6–17 years, this is plausible enough.

All sera from 58 patients with infectious mononucleosis examined had EBV antibody. The geometric mean titre (209·9) in this group was much higher than that in the general population (37·4). Rising titre and seroconversion could be detected in some cases of clinical infectious mononucleosis when serum samples were collected at the right time (Table 4). Because of the long incubation period and relatively mild onset in infectious mononucleosis, this is difficult to estimate. EBV-carrying cell lines were established with relative ease (24–34 days latent period) from 8 of the 12 cases and roughly half of the specimens collected.

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