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Complement-fixing Antibodies Reactive with Epstein–Barr Virus in Sera of Marmosets and Prosimians (37869)

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The widespread distribution of complement-fixing (CF) antibodies reactive with Epstein–Barr virus (EBV) among chimpanzees and Old World monkeys has been reported previously (1). These findings were confirmed by a number of investigators (2–5). However, in contrast to the prevalence of EBV-related antibodies in these nonhuman primates, two groups of investigators (4, 5) failed to detect such antibodies by immunofluorescence (IF) in sera of prosimians or New World monkeys.

Subsequently, Miller *et al.* (6) demonstrated that leukocytes of marmosets and squirrel monkeys are susceptible to *in vitro* infection with EBV. These findings suggested that these lower primates could be susceptible to infection by EBV or a closely related agent. The results of the present studies indicate that this may indeed be the case.

Materials and Methods. Sera. Marmoset sera were obtained from imported and colony-born animals by one of the authors (D. L) and also were kindly made available by Drs. L. Wolfe and F. Deinhardt, Rush–Presbyterian–St. Lukes Medical Center, Chicago, IL. Prosimian sera were provided by Dr. H. Rabin, Bionetics Research Laboratories, Kensington, MD. Some of these sera were used following prolonged storage at −20°, while some were collected during this study and were tested prior to storage. All sera were heated at 56° for 30 min before use. The species, source, and number of sera tested are listed in Tables I and II. All sera were tested for anticomplementary activity. Sera that were anticomplementary at dilutions of 1:16 or greater were excluded from this study.

Complement-fixation tests. The microtiter procedure using 1.6–1.8 U of guinea pig complement was used (7). Crude antigens were prepared by three cycles of freezing and thawing and sonic disruption of a 25% cell suspension in veronal-buffered saline, pH 7.4 (VBS). The cell extracts were centrifuged at 6500*g* for 15 min, and the supernates were stored at 4° following addition of 0.005 *M* sodium azide. Crude EBV antigens were prepared from an EBV-infected human lymphoid cell line "AV" (8). Sucrose-gradient-purified EBV antigens derived from culture fluids of a Burkitt lymphoma cell line P_3HR-1 were kindly furnished by Dr. K. Traul, through a contract by the special Virus Cancer Program, NCI. Two crude control antigen preparations were included in all tests. They were extracted from two human lymphoid cell lines: "MOLT-4" (9) kindly provided by Dr. Minawada, Roswell Park Memorial Institute, Buffalo, NY, or "CCRF-CEM" (10) made available by Dr. Foley, Children's Cancer Research Foundation, Boston, MA. Both of these cell lines appear to be free of EBV antigens or viral DNA [(9) and unpublished observations]. Herpes simplex virus (HSV) type I antigens were prepared from extracts of virus-infected baby-hamster kidney cells in the same manner as the lymphoid-cell antigens. Uninfected BHK cells served as control antigens. Between 2 and 4 U of viral antigens and an equivalent dilution of control antigens were used. The diluent consisted of VBS with 0.1% gelatin. The lowest dilution of serum to be tested was 1:4 or 1:8. All appropriate controls were included in each test.

Absorption studies. Pellets of 3–5 × 10^8 EBV-containing AV cells, virus-free

TABLE I. Imported Simians.

Species	Sera collected		
	Source	Time after arrival	No.
Sanguinus oedipus (cotton-topped marmoset)	Rush[a]	1 month–4 years	
S. fuscicollis (white-lipped marmoset)	Rush	1 month–4 years	108
S. nigricollis (white-lipped marmoset)	Rush	1 month–4 years	
S. mystax (moustached marmoset)	BoB[b]	2–3 weeks	49
Galago crassicaudatus	BRL[c]	unknown	6
Lemur macaco			9

[a] Rush–Presbyterian–St. Lukes Medical Center.
[b] Bureau of Biologics, FDA.
[c] Bionetics Research Laboratories.

MOLT-4, or CCRF-CEM cells were washed three times in VBS. The packed cells were fixed with 10 ml of acetone at room temperature (3). The fixed cells were washed in VBS and aliquots containing 10^8 cells were pelleted. One milliliter of serum diluted 1:8 was added to each pellet, and the mixtures were placed at 37° for 1 hr and 4° for 18–24 hr. This was followed by a second cycle of absorption with fresh cell pellets.

Immunofluorescence test. Acetone-fixed P_3HR-1 or AV cell smears were employed in the indirect test. Sera were tested at a 1:4 dilution. Fluoresceinisothiocyanate-labeled goat antisera to human or rhesus IgG (Hyland Lab) were used at a 1:8 dilution. All appropriate controls were included in each test.

Results. The results obtained by CF tests are summarized in Table III. Of 200 marmoset sera, 63 (32%) were anticomplementary (AC) at a 1:16 dilution and were excluded from this study. The AC activity was lowest among sera from colony-born marmosets and appeared to be unrelated to prolonged frozen storage of sera. None of the sera tested reacted with either of the two control antigens. The percentage of sera reactive with both crude or purified EBV antigens varied from 23 to 64%. The antibody titers ranged from 1:8 to 1:1024 and the geometric mean values were 1:33–1:93. All of the 5 Galago sera had detectable antibodies and 2 of 8 lemur sera had titers of 1:16.

The specificity of the CF reactivity observed was determined by absorption experiments. Representative results obtained with two marmoset sera are summarized in Table IV. Following two absorptions with EBV-positive, acetone-fixed AV cells, the antibody titers to EBV were reduced eightfold in both sera, while absorptions with virus-free cells had no effect on the EBV reactivity. On the other hand, none of these absorptions had any effect on the reactivity of serum 181 with HSV antigens, which adds further evidence for the specificity of the absorption procedures.

It was of interest to correlate the data obtained by CF reactions with immunofluorescence tests. Repeated attempts using varying dilutions of labeled anti-human and anti-rhesus globulin failed to detect EBV-reactive antibodies in any of the sera under study. These negative results are in agreement with published reports (4, 5). In an attempt to discover the possible cause for the negative results obtained in the IF tests, we examined the cross-reactivity of marmoset sera with

TABLE II. Colony-born Marmosets.

Species	Source	No.	Birth dates	Bleeding dates after birth
White-lipped marmoset	Rush	35	From 6/68	3 weeks–2 years
Cotton-topped marmoset	Rush		to 5/70	
Moustached	BoB	8	From 11/69	2–3 years
			to 2/71	

TABLE III. Complement-Fixing Antibodies to Epstein-Barr Virus in Sera of Marmosets and Prosimians.

Species	Source		Total No.	No. sera AC[a]	No. positive/ No. tested	Titer range[b]	Geometric mean titer[b]
Marmoset	Import	BoB	49	24	16/25	8–128	33
Marmoset	Import	Rush	108	34	20/74	8–1024	56
Marmoset	Colony-born	Bob	8	0	5/8	32–256	93
Marmoset	Colony-born	Rush	35	5	7/30	32–256	72
Galago	Import	BRL	6	1	5/5	16–64	28
Lemur	Import	BRL	9	1	2/8	16	16

[a] Sera anticomplementary at dilution of 1:16 or greater.
[b] Reciprocal of serum dilution.

the anti-human and anti-rhesus globulins used in the IF test. The results of repeated immunoelectrophoresis tests demonstrated a weak and partial precipitation of marmoset sera with anti-human and anti-rhesus globulin in the IgG region. These findings are in agreement with the results published by Williams (11).

Discussion. Complement-fixing antibodies reactive with crude and purified EBV antigens were demonstrated in significant titers in 23–64% of sera from imported and colony-born marmosets and prosimians. The number of prosimians and marmosets born in our own colony was too small for a valid calculation of antibody distribution.

The specificity of the serologic reactions was demonstrated by: (a) absence of reactivity with EBV-free control antigens in the 150 sera tested, and (b) absorption studies with two cycles of absorptions with EBV-infected, acetone-fixed cells resulted in an eightfold reduction of EBV-reactive antibodies without effect on antibodies reactive with HSV.

Conversely, absorptions with 2 EBV-free cell lines had no effect on the titers to either viral antigens.

The presence of low levels of HSV-reactive antibodies in marmoset sera is probably a reflection of past infection with *Herpes tamarinus* which has minor antigenic cross-reactivity with HSV (12).

In agreement with previous reports (4, 5), we were unable to demonstrate EBV-reactive antibodies in marmosets or prosimian sera by indirect IF tests using fluorescein-conjugated antisera to human or rhesus IgG. This may be due to the weak cross-reactivity of marmoset IgG with anti-IgG of higher primates detected by immunoelectrophoresis. Prosimians indeed showed no detectable precipitation with anti-human IgG in the studies reported by Williams (11).

It should be noted that Wolfe *et al.* (13) detected EBV antibodies by indirect IF tests in experimentally infected, immature marmosets; however, they failed to indicate whether they used labeled antiserum to homologous or heterologous IgG.

The results of the present study together with our earlier report (1) indicate a widespread distribution of viruses antigenically related to EBV among the higher and lower

TABLE IV. Specific Absorption of EBV Antibodies in Marmoset Sera.

Marmoset serum No.	Test antigens	Antibody titers[a]			
			Absorbed with		
		Unabsorbed	MOLT cells	CCRF CEM cells	AV cells
181	EBV	128	128	128	16
	HSV	32	32	32	32
289	EBV	256	256	256	32
	HSV	<8	<8	<8	<8

[a] Reciprocal of serum dilution.

nonhuman primates. The marmosets imported by us were bled within 1–3 weeks of arrival following 4 days in transit from Peru. No reliable information is available regarding the conditions of caging and care and the period of captivity following trapping. Nevertheless, 64% of 25 animals had significant titers of EBV-reactive antibodies. It should be noted that 51% of 49 sera were anticomplementary, possibly due to the high rate of parasitemia among these unconditioned animals.

Additional studies are needed to determine whether these animals are infected in their natural habitat. It will be of interest to isolate the marmoset agent either from a spontaneously transformed marmoset lymphoid cell line or from oral excretions.

The susceptibility of marmosets and prosimians to EBV-related viruses suggests that these animals may provide useful models for experimental EBV infection. However, care must be taken to avoid possible activation of an endogenous agent.

Summary. Complement-fixing antibodies reactive with Epstein–Barr (EBV) antigens were detected in 35% of 137 marmoset sera tested. The incidence of these antibodies ranged from 23 to 64% depending on the cohort of imported or colony-born marmosets. Similar antibodies were present in sera of 7 of 13 prosimians. The specificity of the serologic reaction was demonstrated by absorption experiments. Attempts to demonstrate EBV-reactive antibodies by indirect immunofluorescence with labeled antisera to human or rhesus IgG were unsuccessful. The results suggest that these lower primates may be susceptible to experimental EBV infection and could provide a model for the study of the pathogenicity of this virus.

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