type of sequence organization is similar to that of the exchangeable cassettes that are responsible for antigenic switching and other types of phase variation in microorganisms (*17*). It would not be surprising if some sort of cassette-switching mechanism were responsible for exchanging these divergent gene segments between strains or species, or both. The evolutionary mechanism for divergence in concert, however, remains unknown and may involve a hypervariability-generating system.

Studies are presently directed toward the biological role or roles of these groupings. Group-specific differences in the expression of subsets of virulence factors or other extracellular proteins could be related to differences in disease patterns. Group-specific differences in the expression of colonization factors could be related to interstrain interference with colonization (*1*) and to differences in colonization site preference. Indeed, we have observed that the vast majority of menstrual toxic shock strains belong to *agr* group III and are characterized by a coherent overall biotype (*12*). This finding could reflect a tissue tropism for the human vaginal mucosa; however, it is inconsistent with the fact that many surface proteins, presumably including colonization factors, are down-regulated by *agr* (*18*). Finally, ligand-based inhibition of virulence or colonization factor expression, or both, could represent the basis of a therapeutic or prophylactic initiative that may not be limited to staphylococci.

REFERENCES AND NOTES

1. H. R. Shinefield *et al.*, *Ann. N.Y. Acad. Sci.* **236**, 444 (1974).
2. W. C. Noble and J. A. Willie, *Br. J. Exp. Pathol.* **61**, 339 (1980); S. E. Holm and E. Grahn, *Scand. J. Infect. Dis. Suppl.* **39**, 73 (1983); K. H. Wilson and J. N. Sheagren, *J. Infect. Dis.* **147**, 733 (1983); M. C. Barc, P. Bourlioux, H. Boureau, F. Nerbone, E. Wasconcellos da Costa, *Can. J. Microbiol.* **32**, 751 (1986).
3. H. L. Peng, R. P. Novick, B. Kreiswirth, J. Kornblum, P. Schlievert, *J. Bacteriol.* **170**, 4365 (1988); L. Janzon, S. Löfdahl, S. Arvidson, *Mol. Gen. Genet.* **219**, 480 (1989).
4. R. P. Novick *et al.*, *Mol. Gen. Genet.* **248**, 446 (1995).
5. R. P. Novick *et al.*, *EMBO J.* **12**, 3967 (1993).
6. L. Janzon and S. Arvidson, *ibid.* **9**, 1391 (1990); E. Morfeldt, D. Taylor, A. von Gabain, S. Arvidson, *ibid.* **14**, 4569 (1995).
7. G. Ji, R. Beavis, R. P. Novick, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 12055 (1995).
8. ———, data not shown.
9. *Staphylococcus aureus* strains used: RN6390B (*5*), RN6596 [American Type Culture Collection (ATCC) 27733], RN7690 (V8), SA502A (ATCC27217), RN6923, and RN6925 (methicillin-resistant clinical isolates); and RN7843, RN8462, and RN8463 (clinical toxic shock syndrome isolates). PCR products were prepared in which chromosomal DNAs from these strains were used as templates and oligonucleotides flanking the *agrBDC* of RN6390B (*4*) were used as primers. For strains RN7690, RN6923, RN6925, and RN8462, the PCR products were directly sequenced with an automated DNA sequencer (Molecular Dynamics), and sequencing was continued with the primer walking strategy. For SA502A and RN8463, the PCR fragments were cloned into plasmid pRN5548 (*5*) and sequenced. *Staphylococcus lugdunensis* RN8160 (*10*) *agrBD* strains were constructed by cloning a PCR product with oligonucleotides flanking the *agrBD* genes as primers and chromosomal DNA as template into pRN5548. pRN5548-RN6390B *agrBD* was constructed as described (*7*). The *agrBD* genes of SA502A and RN8463 were cloned by deleting *agrC* from pRN5548-*agrBDC* constructs. Plasmids used in Table 1: pRN5548-RN6390B *agrD* (*7*) and pRN6441-RN6390B *agrB* were constructed by cloning a Cla I fragment of plasmid pRN6912 (*7*) into pRN6441 (*4*); for other strains: pRN5548-*agrD* plasmids were constructed by in-frame internal deletion of *agrB* genes from pRN5548-*agrBD* plasmids; pRN6441-*agrB* plasmids were constructed by first deleting the *agrD* genes from pRN5548-*agrBD* plasmids, the resulting plasmids were then digested with Cla I, and the DNA fragments containing *agrD* were cloned into pRN6441. All plasmids were transformed into *S. aureus* RN7667 [RN6911(pI524) (*7*)].
10. F. Vandenesch, S. J. Projan, B. Kreiswirth, J. Etienne, R. P. Novick, *FEMS Microbiol. Lett.* **111**, 115 (1993); Y, Benlito, B. Donvito, J. Etienne, F. Vandenesch, *8th International Symposium on Staphylococci and Staphylococcal Infections*, abstract P124, Aix-Les-Bains, France, 23 to 26 June 1996 (Socíeté Française de Microbiologie, 1996).
11. G. Ji, R. Beavis, R. P. Novick, data not shown.
12. G. Ji and R. Novick, unpublished data.
13. R. Beavis, T. Muri, G. Ji, R. P. Novick, unpublished data.
14. L. S. Håvarstein, D. B. Diep, I. F. Nes, *Mol. Microbiol.* **16**, 229 (1995).
15. A. Pedraza and R. Novick, unpublished data.
16. G. Lina *et al.*, unpublished data.
17. B. Wilske *et al.*, *Res. Microbiol.* **143**, 583 (1992); J. Hicks, J. N. Strathern, A. J. S. Klar, *Nature* **282**, 478 (1979); G. L. Murphy and J. G. Cannon, *Bioessays* **9**, 7 (1988).
18. J. Kornblum, B. N. Kreiswirth, S. J. Projan, H. Ross, R. P. Novick, *Molecular Biology of the Staphylococci*, R. P. Novick, Ed (VCH, New York, 1990), chap. 28; C. Wolz, D. McDevitt, T. J. Foster, A. L. Cheung, *Infect. Immun.* **64**, 3142 (1996).
19. The autoinducing peptide from RN6390B was purified as described (*7*). For others, the peptides were purified from the culture supernatants of RN7667 cells containing cloned *agrBD* genes of SA502A, or RN8463, or *S. lugdunensis* grown in dialyzed tryptophan assay medium (*7*) plus L-tryptophan (50 μg/ml) and 2-(2-carboxyphenyl)benzoyl-6-aminopenicillanic acid (5 μg/ml) starting at 2×10^9 cells/ml. After 6-hour growth, cells were removed by centrifugation and the supernatant was filtered (0.22-μm filter), boiled for 10 min, lyophilized, and suspended in solution A (2.5% acetonitrile plus 0.1% trifluoroacetic acid). This material was loaded onto a Sephasil C18 (Pharmacia) column (3 cm × 5 cm), washed once with solution A, once with solution B (15% acetonitrile plus 0.1% trifluoroacetic acid), and eluted with solution C (40% acetonitrile plus 0.1% trifluoroacetic acid). The eluted material was lyophilized, suspended in 20 mM tris-HCl buffer (pH 7.5), and filtered through a Centricon 3 filter (Amicon). The filtrate was then loaded onto a high-performance liquid chromatography C-18 column and eluted with an acetonitrile gradient (16 to 32%) at 0.2% acetonitrile per minute. Fractions with activity were pooled, lyophilized, suspended in 20 mM tris-HCl buffer (pH 7.5), and analyzed by matrix-assisted laser desorption/ionization–mass spectrometry [F. Hillenkamp, M. Karas, R. C. Beavis, B. T. Chait, *Anal. Chem.* **63**, 1193A (1991) (*7*)], and the amino acid sequence was determined by a Perkin-Elmer Procise Edman Sequencer.

20. We thank H. F. Ross for discussion and critical reading of this manuscript. Supported by National Institutes of Health grant R01-A130138 to R.P.N.

23 December 1996; accepted 2 May 1997

An Animal Model for Acute and Persistent Epstein-Barr Virus Infection

Amir Moghaddam, Michael Rosenzweig, David Lee-Parritz, Bethany Annis, R. Paul Johnson, Fred Wang*

Epstein-Barr virus (EBV) is a human lymphocryptovirus that causes infectious mononucleosis, persists asymptomatically for life in nearly all adults, and is associated with the development of B cell lymphomas and nasopharyngeal carcinomas. A highly similar rhesus lymphocryptovirus naturally endemic in rhesus monkeys was used to orally infect naïve animals from a pathogen-free colony. This animal model reproduced key aspects of human EBV infection, including oral transmission, atypical lymphocytosis, lymphadenopathy, activation of $CD23^+$ peripheral blood B cells, sustained serologic responses to lytic and latent EBV antigens, latent infection in the peripheral blood, and virus persistence in oropharyngeal secretions. This system may be useful for studying the pathogenesis, prevention, and treatment of EBV infection and associated oncogenesis.

Despite progress in basic and clinical Epstein-Barr virus (EBV) research, the absence of a suitable animal model for EBV infection continues to impede studies of viral pathogenesis, vaccine development, and therapeutics. EBV infection is typically initiated by transmission in oral secretions, virus replication in the oropharynx, and infection of peripheral blood B lymphocytes (*1*). Primary EBV infection is the most common cause of infectious mononucleosis (*2*). Thereafter, EBV persists as a latent infection in a small fraction of B lymphocytes and replicates sporadically at low levels in the oropharynx (*1*). Virus reactivation and shedding in oral secretions enables completion of the virus life cycle by infection of naïve hosts. Latent EBV infection is generally asymptomatic but can lead to polyclonal EBV-infected B cell lymphoproliferations and lymphomas when individuals are immunosuppressed

because of congenital immunodeficiencies, after transplantation, or by human immunodeficiency virus (HIV) infection (*3*). Persistent EBV infection is also associated with the development of Burkitt's lymphoma, nasopharyngeal carcinoma, and certain types of Hodgkin's disease (*3*).

The growth-transforming properties of EBV can be demonstrated by the continuous proliferation of EBV-infected human B cells in tissue culture or when injected into mice with severe combined immunodeficiency (*4*). Parenteral inoculation of cotton-top tamarins with EBV can also result in acute polyclonal proliferation of infected B lymphocytes (*5*). However, animals that do not die from lymphoproliferative disease do not become persistently or latently infected with EBV. Therefore, there is no animal model for the persistent or latent EBV infection that affects almost all humans.

Most Old World nonhuman primates, such as macaques, baboons, chimpanzees, and apes, are infected with herpes viruses of the same subgroup [lymphocryptovirus (LCV)] as EBV (*6*, *7*) and could provide a model for EBV infection. The genomes of all LCVs are colinear; encode highly homologous structural proteins; and replicate by similar, interchangeable mechanisms (*8–10*). LCVs can immortalize B cells from their natural host in vitro (*11*), and they encode a similar repertoire of viral proteins that are homologous to each of the nuclear (EBNA, EBV nuclear antigen) and membrane (LMP, latent membrane protein) proteins expressed during latent EBV infection. LCV EBNA-1, EBNA-2, LMP1, and LMP2A homologs interact with the viral latent origin of replication, CBF1/RBP-Jk, TRAF3, and Src kinases, respectively, which are the same viral and cell targets as those of EBV EBNA-1, EBNA-2, LMP1, and LMP2A (*10*, *12–14*). Like humans, almost all Old World primates are naturally infected with their endogenous LCV by adulthood, have LCV-infected B cells in the peripheral blood, and maintain serum antibody responses for life (*11*). These antibodies cross-react with EBV antigens and can be used to identify LCV infection (*15–17*). Simian immunodeficiency virus (SIV)–infected monkeys develop LCV-infected B cell lymphomas that are similar to the EBV-infected B cell lymphomas that occur in acquired immunodeficiency syndrome (AIDS) patients or transplant recipients (*18*). Earlier attempts to infect Old World primates with EBV were unsuccessful (*7*, *19*, *20*), possibly because of cross-reactive immunity as these animals were often not screened for previous simian LCV infection. Also, EBV does not immortalize rhesus B cells in vitro (*21*), and this may have contributed to the failure to persistently infect nonhuman Old World primates with EBV.

Because of the need for better animal models of human EBV infection, we explored whether rhesus monkeys can be experimentally infected with rhesus LCV and to what extent experimental infection recapitulates key aspects of EBV infection. As expected, serologic testing for cross-reactive antibodies to EBV identified ubiquitous rhesus LCV infection in the conventional rhesus monkey colony at the New England Regional Primate Research Center (NERPRC). In contrast, serologic testing indicat-

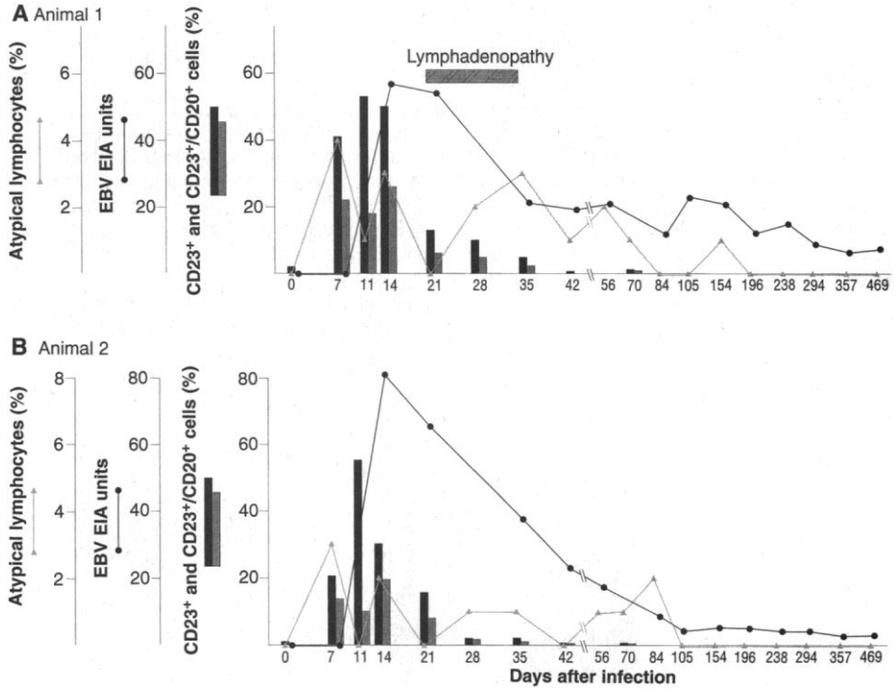

Fig. 1. Atypical lymphocytosis, lymphadenopathy, CD23-positive lymphocytes, and serologic responses to experimental rhesus LCV infection of rhesus monkeys. Atypical lymphocytes in the peripheral blood are expressed as the percent of white blood cells. The period of detectable lymphadenopathy is shown for animal 1. Pharyngitis and splenomegaly were not evident on physical examination. The percentages of lymphocytes positive for CD23 or positive for both CD23 and CD20 are shown as solid and grey bars, respectively. Assays for CD23 expression were not performed on day 56 or after day 84. The titers of antibodies reactive with EBV are shown in relative EBV EIA units. Rhesus LCV was obtained from LCL8664 cells induced for viral replication by transfection with an EBV BZLF1 expression vector as previously described (*10*, *22*). Complete blood counts were performed on an automated Coulter counter, and atypical lymphocytosis was confirmed by visual examination of the blood smear in a blinded fashion. Atypical lymphocytes were not observed in routine smears of blood obtained before infection. Cell surface CD23 and CD20 expression was determined by staining of whole blood with fluoroscein isothiocyanate–conjugated, CD23-specific, monoclonal antibody EBVCS4 [kindly provided by B. Sugden (*23*)] and phycoerythrin-conjugated, CD20-specific, monoclonal antibody Leu16 (Becton-Dickinson), followed by fixation and blood lysis. Dual-color surface fluorescence was analyzed on a Becton-Dickinson FACScan, and at least 20,000 events were recorded for each population. EIA microtiter wells were coated with EBV concentrated by centrifugation from cell-free supernatants of P3HR-1 clone 16 cells (*30*) induced for viral replication with phorbol ester–sodium butyrate and fractionated by Sepharose 4CL-B chromatography as described (*31*). Sera were assayed at two dilutions in duplicates, developed with an antiserum to human immunoglobulin G (IgG) conjugated to horseradish peroxidase and *o*-phenylenediamine dihydrochloride, and read at an optical density of 450 nm. Titers were determined with a commercial software program (Bio-Rad Microplate Manager v4.0) referenced to a standard curve with an EBV-positive human serum (VCA titer 1:20,000) arbitrarily designated as 10,000 EBV EIA units.

A. Moghaddam, B. Annis, F. Wang, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA.
M. Rosenzweig, Division of Immunology, New England Regional Primate Research Center, Harvard Medical School, 1 Pine Hill Drive, Southborough, MA 01172, USA.
D. Lee-Parritz, Division of Primate Resources, New England Regional Primate Research Center, Harvard Medical School, 1 Pine Hill Drive, Southborough, MA 01172, USA.
R. P. Johnson, Division of Immunology, New England Regional Primate Research Center, Harvard Medical School, 1 Pine Hill Drive, Southborough, MA 01172, USA, and AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA 02129, USA.
*To whom correspondence should be addressed. E-mail: fwang@rics.bwh.harvard.edu

ed that rhesus LCV infection was less prevalent in the NERPRC's specific pathogen-free (SPF) colony of rhesus monkeys derived from hand-reared founder animals and kept free of herpes B virus, simian T cell leukemia virus type 1, simian type D retrovirus, and SIV infection. Two rhesus monkeys naïve for rhesus LCV were selected from the SPF colony after serial EBV serologic testing, and these animals were inoculated with 10^6 transforming units of rhesus LCV (*22*) by topical, nontraumatic application throughout the oral cavity.

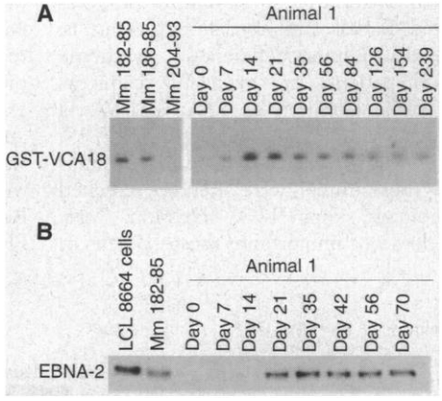

Fig. 2. Serum antibody response to viral capsid antigen VCAp18 (**A**) and latent infection nuclear protein EBNA-2 (**B**) after experimental rhesus LCV infection. (A) Serum antibodies to VCAp18 were detected by protein immunoblotting with a glutathione-S-transferase (GST)–VCAp18 fusion protein (GST-VCA18). Sera from two EBV-seropositive animals in the conventional colony (Mm 182-85 and Mm 186-85) were used as positive controls, and serum from an EBV-seronegative animal in the SPF colony (Mm 204-93) was used as a negative control. The GST-VCAp18 fusion protein migrates with a relative molecular mass of approximately 45 kD. (B) Serum antibodies to rhesus LCV EBNA-2 were detected by immunoprecipitation with rhesus sera and protein immunoblotting for EBNA-2. Mm 182-85 serum (lane 2) and lysate representing 500,000 LCL 8664 cells (lane 1) were used as positive controls. Rhesus LCV EBNA-2 migrates with a relative molecular mass of 100 kD. The GST-VCAp18 fusion protein was made by fusion of the EBV BFRF3 open reading frame with the NH_2-terminus of GST, expression in bacteria, and purification with glutathione-Sepharose beads. GST-VCAp18 protein immunoblots were incubated in a multichannel apparatus (Bio-Rad) with rhesus serum diluted at 1:100 in 5% milk and phosphate-buffered saline (PBS) with 20% GST control bacterial lysate for blocking. Blots were developed with HRP-conjugated antiserum to human IgG and chemiluminescence. Immunoprecipitations for rhesus LCV EBNA-2 were performed by incubation of 3 μl of rhesus serum in a lysate of 2 million LCL 8664 cells in PBS with 1% deoxycholate and 0.1% SDS, precipitation of immune complexes with protein G–Sepharose, and protein immunoblotting for EBNA-2 in the immunoprecipitates with the PE2 monoclonal antibody (*32*).

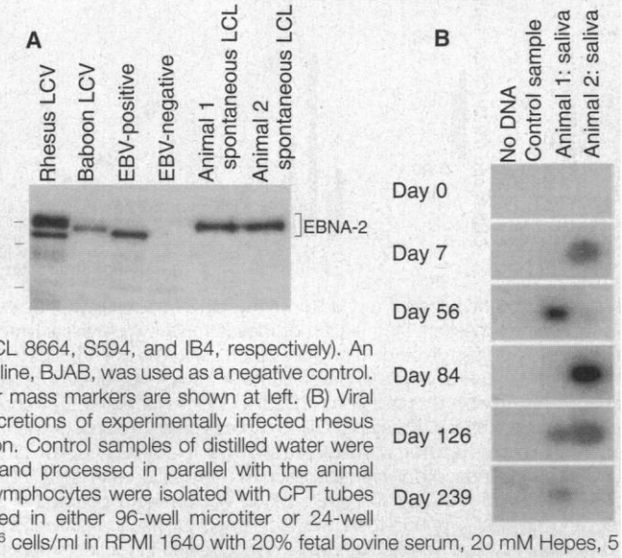

Fig. 3. Rhesus LCV persistence in peripheral blood (**A**) and oropharyngeal (**B**) compartments. (A) EBNA-2 protein immunoblot of spontaneous B cell lines recovered from the peripheral blood of rhesus LCV–infected animals confirms rhesus LCV infection. EBNA-2 from simian LCV and EBV can be distinguished by the different relative molecular mass in rhesus LCV, baboon LCV, and EBV-infected cell lines (LCL 8664, S594, and IB4, respectively). An EBV-negative B lymphoma cell line, BJAB, was used as a negative control. 92-, 66-, and 45-kD molecular mass markers are shown at left. (B) Viral DNA was detected in oral secretions of experimentally infected rhesus macaques by PCR amplification. Control samples of distilled water were harvested at each time point and processed in parallel with the animal specimens. Peripheral blood lymphocytes were isolated with CPT tubes (Becton-Dickinson) and cultured in either 96-well microtiter or 24-well plates at a concentration of 10^6 cells/ml in RPMI 1640 with 20% fetal bovine serum, 20 mM Hepes, 5 mM 2-mercaptoethanol, and cyclosporin A (0.5 μg/ml). Oral secretions were collected from anesthetized animals by the addition of 1 ml of distilled water to the oral cavity and aspiration of oral fluids. Control and animal samples were boiled for 5 min, digested with proteinase K (200 μg/ml) for 30 min at 55°C, and boiled for an additional 5 min; 2 μl of sample was used for PCR amplification of a 120–base pair (bp) fragment with primers for the major internal repeat region of rhesus LCV. The rhesus LCV major internal repeat sequences were obtained by PCR amplification of LCL 8664 genomic DNA with EBV Bam HI W primers at low stringency. The rhesus LCV PCR DNA sequence is 90% homologous with that of EBV Bam HI W. Rhesus LCV primers RBW1 (5′-CCAGAGGTAAGTGGACTT-3′) and RBW2 (5′-TGCCGGCGCCTTCTCAGG-3′) derived from this region specifically amplify a 120-bp fragment from LCL 8664 at an annealing temperature of 60°C but not from EBV-negative human B lymphoma cells or EBV-infected B cells. PCR products were transferred by Southern (DNA) blotting and probed with another internal rhesus LCV radiolabeled oligonucleotide (RBW-P; 5′-CTCTGCTCAGCCCACC-3′) for specificity.

Acute responses that are characteristic of human EBV infection became evident on physical examination, on microscopy of peripheral blood smears, and on flow cytometry analysis (FACS) of peripheral blood lymphocytes. Axillary and inguinal lymphadenopathy was evident in animal 1 between 3 and 5 weeks after infection. Both animals developed 1 to 3% atypical lymphocytes in the peripheral blood at 1 week after infection, and the atypical lymphocytosis resolved after 10 weeks (Fig. 1). Both animals also developed a dramatic increase in peripheral blood $CD23^+$ cells detected by FACS as early as 7 days after oral inoculation (Fig. 1). CD23 is an activation marker induced on B cells by EBV infection in vitro (*23*, *24*), and $CD23^+$ B cells and monocytes and macrophages are frequently detected in patients with acute infectious mononucleosis (*25*). The $CD23^+$ response in experimentally infected animals peaked on days 11 to 14, with 50 to 56% of the peripheral blood lymphocytes being positive for CD23, and the response resolved to near baseline levels of fewer than 5% within 3 to 4 weeks after infection. Double immunostaining with the B cell marker CD20 revealed that up to 60% of the $CD23^+$ cells were activated B cells (Fig. 1). The remaining $CD23^+/CD20^-$ cells had an increased size and granularity that were consistent with monocytes (*26*). Thus, oral inoculation of naïve rhesus monkeys with rhesus LCV resulted in atypical lymphocytosis, lymphadenopathy, and an activated $CD23^+$ B cell response that are characteristic of primary EBV infection and infectious mononucleosis.

Enzyme immunoassays (EIAs) with EBV antigen preparations were used to detect an antibody response to LCV infection. Both animals developed high-level antibody responses that peaked at day 14 (Fig. 1). An antibody level of 2 to 7 EIA units persisted in these animals for over 1.5 years after infection. This antibody level was comparable to the mean antibody titer in randomly selected, naturally infected LCV-seropositive animals (mean titer of 3.0 EIA units in 13 random seropositive animals versus a mean titer of 0.9 EIA units in 8 random seronegative animals). Antibodies to specific viral antigens were analyzed further in animal 1. Antibodies to the EBV capsid antigen p18 (VCAp18) were detected by protein immunoblotting as early as day 7, with a peak response on days 14 to 21, and persisted at a lower level for at least 1.5 years after infection (Fig. 2A). Antibodies to EBNA-2, a rhesus LCV gene product, were detected by immunoprecipitation. A trace amount of EBNA-2 antibody could be detect-

ed on day 14, with readily detectable EBNA-2 antibodies being present by day 21 after infection (Fig. 2B). Because EBNA-2 is expressed in latently infected, immortalized B cells but is not expressed in epithelial cells (*1*), the induction of serum antibodies specific for EBNA-2 is consistent with the expression of viral latent infection genes in the B cell compartment.

Experimental infection resulted in persistence of rhesus LCV in the peripheral blood and in shedding of virus in oral secretions, which is similar to the human infection pattern. Rhesus LCV-infected B cell lines were recovered when peripheral blood lymphocytes from the infected animals were cultured in vitro. No rhesus LCV-infected B cells were obtained from two separate blood samples of either animal before experimental infection. Rhesus LCV-infected B cells were recovered from animal 1 at 21, 84, 206, 238, and 525 days after infection and from animal 2 at 21 days after infection. Rhesus LCV infection was confirmed in these cell lines by protein immunoblotting for EBNA-2 expression (representative cell lines from animals 1 and 2 are shown in Fig. 3A). Virus persistence in the oral secretions was detected by polymerase chain reaction (PCR) amplification of rhesus LCV DNA for several weeks after experimental infection (Fig. 3B). Viral DNA was detectable intermittently in the oropharynx of both animals for over 1.5 years of observation. The intermittent shedding of viral DNA as detected by PCR in the experimentally infected animals is similar to the episodic EBV DNA shedding seen in most healthy EBV-seropositive humans (*27*). Both animals have been followed for 23 months after infection and have remained healthy with normal weight gain, sexual maturity, and no evidence of tumors.

Immunity induced by primary EBV infection usually protects humans from subsequent episodes of infectious mononucleosis. We rechallenged animal 1 with a second dose of virus at day 525 to determine whether the primary LCV infection provided any protection from reinfection. A comparable oral dose of 10^6 transforming units did not result in lymphadenopathy or atypical lymphocytosis. The number of peripheral blood $CD23^+$ cells varied from 3 to 8% [which is not different from the baseline before rechallenge (Table 1)]. EBV antibody titers did not change in the 5 weeks after rechallenge (Table 1). Thus, the lymphadenopathy, atypical lymphocytosis, and increase in $CD23^+$ cells were specific for primary infection, and primary infection appeared to protect against rechallenge. Whether experimental infection completely blocks transient or long-term colonization of oral mucosal cells or the peripheral blood compartment by rechallenge virus remains to be determined.

Table 1. Protection from acute responses during rhesus lymphocryptovirus rechallenge. Animal 1 was orally rechallenged with virus 525 days after primary inoculation and the days after virus rechallenge are shown. The percent of $CD23^+$ and $CD23^+/CD20^+$ cells in the peripheral blood, atypical lymphocytosis and EBV antibody titers were measured as described in Fig. 1. ND, not determined.

Day	$CD23^+$ (%)	$CD23^+/CD20^+$ (%)	Atypical lymphocytes	EBV EIA units
0	3.7	2.4	0	6.8
4	3.6	2.2	0	ND
7	7.7	3.6	0	6.5
14	4.5	3.3	0	5.9
21	8.8	2.9	0	ND
28	7.4	4.9	0	ND
35	5.7	4.5	0	6.5

This study shows that experimental rhesus LCV infection of its natural host is highly analogous to EBV infection of humans, and it opens a new approach to study of the pathogenesis of acute EBV infection, latency, and oncogenesis. Early stages of EBV infection can be studied without waiting for the development of symptoms to identify infected individuals, and the earliest cytokines and immune responses that initiate infectious mononucleosis can be identified. This animal model also provides an experimental system in which the role of specific, latent-infection viral genes in persistence and latency can be investigated. Persistence and latency are as yet poorly understood dynamic states requiring different patterns of latent infection gene expression in different cell compartments in order for the EBV-infected cell to evade immune surveillance (*3*). The EBNA-3B, LMP2A, and EBER latent infection genes are conserved in both human and nonhuman LCV, but their role in EBV infection is incompletely understood (*1*). In vivo infections with genetically modified LCV to address the role of these viral genes in persistence and latency can now go forward. Interactions between HIV, EBV, and the host that result in a high frequency of EBV-induced lymphomas in AIDS patients can be more readily investigated in animals coinfected with SIV and LCV. The role of co-carcinogens in converting persistent EBV infection into nasopharyngeal carcinoma can be directly investigated. Further studies of nonhuman primate LCVs are also required, because these viruses are likely to be inadvertently transmitted to humans through xenotransplanted organs, just as EBV can be transmitted among humans by blood transfusions and transplanted organs (*28*, *29*).

This animal model will also be important for the development of vaccines and therapies against the diverse spectrum of EBV-associated diseases. Vaccines that induce neutralizing antibodies and mucosal immunity may be effective in limiting acute EBV infection. Increasing cellular immunity in normal and immunosuppressed individuals who are latently infected with EBV may reduce the risk of subsequent tumorigenesis. Novel pharmaceutical, biological, and molecular therapies against EBV infection can also now be evaluated in vivo.

REFERENCES AND NOTES

1. E. Kieff, in *Virology*, D. Knipe, B. Fields, P. Howley, Eds. (Raven, Philadelphia, PA, 1996), pp. 2343–2396.
2. G. Henle, W. Henle, V. Diehl, *Proc. Natl. Acad. Sci. U.S.A.* **59**, 94 (1968).
3. A. B. Rickinson and E. Kieff, in *Virology*, D. Knipe, B. Fields, P. Howley, Eds. (Raven, Philadelphia, PA, 1996), pp. 2397–2446.
4. D. E. Mosier *et al.*, *Curr. Top. Microbiol. Immunol.* **166**, 317 (1990).
5. T. Shope, D. Dechairo, G. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2487 (1973).
6. D. V. Ablashi, P. Gerber, J. Easton, *Comp. Immunol. Microbiol. Infect. Dis.* **2**, 229 (1979).
7. A. Frank, W. A. Andiman, G. Miller, *Adv. Cancer Res.* **23**, 171 (1976).
8. M. Heller, P. Gerber, E. Kieff, *J. Virol.* **37**, 698 (1981).
9. J. J. Ryon *et al.*, *ibid.* **67**, 4006 (1993).
10. M. Franken, B. Annis, A. Ali, F. Wang, *ibid.* **69**, 8011 (1995).
11. P. Gerber, S. S. Kalter, G. Schidlovsky, W. J. Peterson, M. D. Daniel, *Int. J. Cancer* **20**, 448 (1977).
12. D. D. Loeb *et al.*, *J. Virol.* **64**, 2876 (1990).
13. P. D. Ling and S. D. Hayward, *ibid.* **69**, 1944 (1995).
14. M. Franken *et al.*, *ibid.* **70**, 7819 (1996).
15. J. A. Levy, S. B. Levy, Y. Hirshaut, G. Kafuko, A. Prince, *Nature* **233**, 559 (1971).
16. V. C. Dunkel, T. W. Pry, G. Henle, W. Henle, *J. Natl. Cancer Inst.* **49**, 435 (1972).
17. S. S. Kalter, R. L. Heberling, J. J. Ratner, *Nature* **238**, 353 (1972).
18. H. Feichtinger *et al.*, *J. Exp. Med.* **176**, 281 (1992).
19. P. Gerber, J. W. Branch, E. N. Rosenblum, *Proc. Soc. Exp. Biol. Med.* **130**, 14 (1969).
20. P. H. Levine *et al.*, *Arch. Virol.* **66**, 341 (1980).
21. A. Moghaddam, J. Koch, B. Annis, F. Wang, in preparation.
22. S. R. Rangan, L. N. Martin, B. E. Bozelka, N. Wang, B. J. Gormus, *Int. J. Cancer* **38**, 425 (1986).
23. C. Kintner and B. Sugden, *Nature* **294**, 458 (1981).
24. D. Thorley-Lawson, L. M. Nadler, A. K. Bhan, R. T. Schooley, *J. Immunol.* **134**, 3007 (1985).
25. S. Furukawa *et al.*, *J. Infect. Dis.* **166**, 691 (1992).
26. Increased size and granularity were determined by increased side and forward light scatter on FACS.
27. Q. Y. Yao, A. B. Rickinson, M. A. Epstein, *Int. J. Cancer* **35**, 35 (1985).
28. C. Alfieri *et al.*, *Blood* **87**, 812 (1996).
29. J. W. Gratama *et al.*, *Transplant Proc.* **21**, 3097 (1989).
30. L. Heston, M. Rabson, N. Brown, G. Miller, *Nature* **295**, 160 (1982).
31. M. D. Daniel *et al.*, *Int. J. Cancer* **41**, 601 (1988).
32. L. Young *et al.*, *N. Engl. J. Med.* **321**, 1080 (1989).

33. Supported by USPHS grants CA68051 and CA65319 and by USPHS grant P51RR00168 to the NERPRC. E. Kieff and R. Desrosiers provided advice. P. Sehgal, E. Roberts, and the NERPRC staff provided assistance with animal care. D. Marks provided assistance in flow cytometry. J. Sample provided a highly inducible P3HR-1 cell line originally derived by G. Miller. All animal care was provided in accordance with institutional guidelines.

3 March 1997-accepted 14 May 1997