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# Differences among Isolates of Simian Hemorrhagic Fever (SHF) Virus (42231)

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*Abstract.* Simian hemorrhagic fever (SHF) virus is a member of the Togaviridae family which currently is unclassified to genus. We have studied the relatedness of four different SHF virus isolates obtained from infected macaque or patas monkeys. Differences were found among isolates in type and severity of disease produced in patas monkeys, cell sensitivity to infection, viral antigens, and levels of specific antibody induced in patas monkeys. Based on these criteria, the four isolates have been grouped in two categories: those producing acute infections in patas monkeys (LVR, P-180) and those producing persistent infections (P-248, P-741). The P-180 isolate induced the most severe disease in experimentally infected patas monkeys, but only occasionally were their infections fatal. Persistently infected patas monkeys were viremic over a period of years, but showed no signs or symptoms of infection. All four isolates were found to be antigenically related by use of enzyme-linked immunosorbent assay (ELISA); the P-248 isolate showing the weakest antigenic relationship. However, none of the four isolates induced cross-neutralizing antibodies in infected patas monkeys. High titers of specific IgG antibody (up to 31,250 as determined by ELISA) were induced in acutely infected patas monkeys (LVR, P-180), but antibody was barely detectable ($\leqslant$50) in persistently infected patas monkeys (P-248, P-741). LVR lytically infected USU-104 cells, patas monkey peritoneal macrophages (PMAC), and rhesus monkey PMAC. The P-180 isolate lytically infected both patas monkey PMAC and rhesus monkey PMAC, but not USU-104 cells. The isolates producing persistent infections (P-248, P-741) lytically infected only rhesus monkey PMAC. These results show that marked differences exist among isolates of SHF virus from naturally infected animals. These differences should be useful in categorizing new isolates. 

Simian hemorrhagic fever (SHF) virus, a togavirus, has a very narrow host range. It does not infect common laboratory animals such as mice, rats, hamsters and guinea pigs, many genera of monkeys, nor man (1). In nature, infection has only been associated with species of African monkeys from three genera, the patas monkey (*Erythrocebus patas*), the African green monkey (*Cercopithecus aethiops*), and baboons (*Papio anuibus* and *Papio cynocephalus*) (2). Little information has been reported concerning the morbidity and mortality associated with SHF virus infections of these species in their natural habitat. Fatal infections of African green monkeys and baboons housed in laboratory colonies have not been reported and only occasionally have captive patas monkeys succumbed from infection. It is unknown whether competition to survive in nature exacerbates the severity of SHF virus infections of these species.

In marked contrast to the limited severity of infections of the natural hosts, SHF virus infections of monkeys of the genus *Macaca* are nearly always fatal. Macaque monkeys generally die, 5 to 14 days after infected, of a hemorrhagic hypovolemic shock syndrome. Defects in homeostasis, increased vascular permeability, disseminated intravascular coagulation, and multiple hemorrhages throughout the organs of their bodies are common characteristics of infection (3). Several devastating epizootics of SHF have occurred in laboratory colonies and primate centers where macaque species have been housed (2, 4–6). Natural epizootics of SHF in macaque monkeys, however, have not been reported, probably because of the geographic separation of the involved species. Macaque monkeys reside in Asia and the naturally infected species in Africa.

Among the natural hosts, SHF virus infections of the patas monkey have been most extensively studied. A low percentage of feral patas monkeys have been shown to be long-term, persistent carriers of SHF virus (2). Persistently infected animals studied in captivity have shown no overt signs or symptoms of



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illness and outwardly are indistinguishable from normal healthy animals. These animals are continuously viremic as blood drawn from them periodically over a number of years has always contained infectious virus. In spite of the covert, asymptomatic nature of their infection, these animals should not be considered harmless, since they have been identified as the source of virus from which a severe epizootic of SHF was iatrogenically initiated in a colony of macaques (2).

The prototype strain of SHF virus, known as LVR, lytically infects and grows to high titer in a cell line established from embryonic rhesus monkey kidney which has been referred to either as MA-104 or USU-104 [(6, 7) M. M. Vincent, personal communication]. Conversely, virus from an asymptomatic, persistently infected patas monkey (P-248) did not produce any discernable cytopathology in USU-104 cells, but produced a nonlytic persistent infection (7). Although a wide variety of other types of primary cells and cell lines have been tested for susceptibility to lytic infection by the P-248 virus isolate, only primary macrophages from the rhesus monkey, *Macaca mulatta,* were susceptible (8). Macrophages from other susceptible species of macaque monkeys may also be sensitive to lytic infection, but have not been tested. Based on these results, we have postulated that the extreme lethality of SHF virus infections of monkeys of the genus Macaca may be related to the sensitivity of their macrophages to infection and lysis (8).

Because biological differences have been found among different isolates of SHF virus, we initiated studies to more completely characterize four different isolates. The antigenic relatedness, susceptibility of different cell types to infection, and the immune responses of patas monkeys to infection with these isolates have been studied. Results of those studies are communicated in this article.

**Materials and Methods.** *Virus isolates.* Four different isolates of SHF virus were used in this study. Two of these isolates have been described previously: LVR 42-0/M6941 (American Type Culture Collection, VR-533, Rockville, Md.), the prototype strain of SHF virus (subsequently referred to as LVR) and P-248, an isolate made in our laboratories from a feral patas monkey which was found to be persistently infected when introduced into the National Institutes of Health (NIH) colony (1, 7). The other two agents were also isolated in our laboratories and have been called P-180 and P-741 (M. Gravell and W. London). The P-180 isolate was made from the spleen of patas monkey No. 180, a monkey which died in 1972 from an infection acquired during an epizootic at the Corbel Facility, Rockville, Maryland, where monkeys from the NIH colony were housed. P-741 was isolated from a rhesus monkey which died of SHF after inoculation with serum from patas monkey No. 741, a feral animal shown by this screening procedure to have an asymptomatic, persistent infection on arrival at NIH. The species of origin of the LVR and P-180 isolates cannot be determined as they were isolated from animals infected in captivity by unknown sources.

*Virus growth, purification, and assay.* The LVR isolate of SHF virus was grown in USU-104 cells as described previously (9). The P-180 isolate was grown in primary peritoneal macrophages (PMAC) from patas monkeys, while the P-248 and P-741 isolates were grown in PMAC from rhesus monkeys. The PMAC were collected and purified by a procedure described previously (8). Approximately 40 $\times 10^7$ rhesus or patas monkey PMAC were suspended in 25 ml of Eagle's minimum essential medium plus 10% fetal bovine serum (EMEM-10) and seeded into 75-cm$^2$ plastic tissue culture flasks (Costar, Cambridge, Mass.). Cultures were incubated at 37°C in an atmosphere of 5% carbon dioxide in air and infected the day after planted with 0.1 to 1 $TCID_{50}$/cell of P-180, P-248, or P-741 virus. Unconcentrated harvests of P-180 virus contained $10^7$ to $10^8$ $TCID_{50}$/ml and similar harvests of P-248 or P-741 contained $10^6$ to $10^7$ $TCID_{50}$/ml.

Harvests of all four viruses were clarified by centrifugation at 2000*g* for 15 min and purified and concentrated by centrifugation in discontinuous and continuous neutral sucrose gradients by a method described previously (9).

Infectivity of the LVR isolates was determined by plaque assay in USU-104 cells (9) and that of the P-180, P-248, and P-741 isolates was determined by a 50% endpoint assay in either primary patas or rhesus monkey PMAC cultures, as appropriate (8).

*Antibody determinations by enzyme-linked*

TABLE I. CHRONIC CARRIERS OF SHF VIRUS

| Patas monkey No. | Sex | Date animal found to be chronic carrier | IgG antibody titer to SHF virus[a] |
|---|---|---|---|
| 209 | Female | 12/30/73 | 6250[b] |
| 226 | Female | 11/04/76 | 6250 |
| 248 | Female | 05/08/74 | 50 |
| 355 | Male | 12/21/74 | 250 |
| 468 | Female | 11/17/75 | ≤10 |
| 470 | Female | 11/17/75 | ≤10 |
| 494 | Female | 10/28/76 | 1250 |
| 553[c] | Female | 02/17/77 | 50 |

[a] Purified LVR strain SHF virus was used as the ELISA antigen.

[b] Reciprocal of the serum dilution yielding a positive ELISA result (≥0.2 $A_{405}$ units above background).

[c] Patas monkey No. 553 died on 03/18/75 of dystocia.

*immunosorbent assay (ELISA).* Titers of antibody to each of the 4 virus isolates were determined by ELISA in 96-well, flat-bottom polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va., Catalog No. 1-223-29). The enzyme–antibody conjugate was rabbit anti-human IgG or anti-human IgM to which alkaline phosphatase was attached (Microbiological Associates, Inc., Walkersville, Md.). The substrate was *p*-nitrophenyl phosphate (1 mg/ml) (Sigma Chemical Co., St. Louis, Mo.). Optimum concentrations of purified virus and enzyme–antibody conjugate for use in tests were determined by block titration. The reagents and methods by which ELISA tests were performed have been described previously (10).

*Virus neutralization.* Titers of neutralizing antibody in sera from patas monkeys which had undergone infection with different isolates of SHF virus were determined as follows: Serial twofold dilutions of serum samples were mixed with an equal volume of challenge virus diluted in EMEM-2. The serum–virus mixtures were incubated for 2 hr at 37°C and then inoculated onto rhesus monkey PMAC in 24-well plates (Costar). Each culture well received 0.2 ml of inoculum containing about 100 $TCID_{50}$ of challenge virus. The inoculum was adsorbed to cells for 1 hr at 37°C and 1 ml of medium (EMEM + 10% fetal bovine serum and 10% normal patas monkey serum) was added per culture well. Cultures were incubated at 37°C in an atmosphere of 5% $CO_2$ in air and read when control cultures inoculated with 100 $TCID_{50}$ of virus showed total cytopathology, usually 3 days after infection.

**Results.** *Antigenic relatedness of different SHF virus isolates.* The titers of IgG antibodies to SHF virus in serum from eight persistently infected patas monkeys were determined by ELISA using purified protype LVR virus as antigen. These animals were known to be chronic carriers of SHF virus from the date indicated in Table I because their serum or plasma contained virus which produced characteristic cytopathology when inoculated into cultures of primary rhesus monkey PMAC (8) or fatal disease when inoculated into susceptible macaque monkeys (1). This result suggested that major antigenic differences might exist between virus present in different animals.

For this reason, an ELISA was used to determine the antigenic relationship of four isolates of SHF virus: LVR, P-180, P-248, and P-741. The origin of these four isolates was briefly described under Materials and Methods. The LVR and P-180 isolates produce an acute infection in patas monkeys and the P-248 and P-741 isolates an asymptomatic long-term persistent infection. The serum antibodies used in these comparisons were obtained from patas monkeys, each of which had undergone an infection with one of the four virus isolates specified. The results of the antigenic comparison of the four virus isolates are shown in Table II. The results show the LVR isolate to be quite closely related antigenically to the P-180 and P-741 isolates, but only weakly related to the P-248 isolate. The P-180 isolate was closely related to LVR, but was more distantly related to the two persistent strains, P-248 and P-741. Unexpectedly, serum from animals which were persistently infected with P-248 or P-741 virus only had low titers of

TABLE II. ANTIGENIC RELATIONSHIP BETWEEN FOUR ISOLATES OF SHF VIRUS

| | Antibody | | | |
|---|---|---|---|---|
| Antigen | LVR | P-180 | P-248 | P-741 |
| LVR | 1250[a] | 1250 | 10 | 50 |
| P-180 | 250 | 1250 | <10 | 50 |
| P-248 | 50 | 50 | 10 | 10 |
| P-741 | 250 | 50 | <10 | 50 |

[a] Reciprocal of the serum dilution yielding a positive ELISA result (≤0.2 $A_{405}$ units above background).

antibody against both the homologous and heterologous isolates. Over the years that sera from these persistently infected animals were periodically tested, they always had low titers of antibody to their homologous virus.

*Antibody titers in persistently infected monkeys.* The fact that the P-248 and P-741 isolates induced only low levels of antibody in infected patas monkeys was enigmatic because some of the persistently infected animals listed in Table I had high titers of antibody to SHF virus. It suggested the possibility that some persistent strains of SHF virus might have the capacity to induce high levels of specific antibody in patas monkeys, while others could induce only low levels. To determine if this postulate could be verified, virus was isolated from patas monkey No. 226 in primary rhesus monkey PMAC. As shown in Table 1, patas monkey No. 226 had an antibody titer of 6250 to LVR antigen. Serum from patas monkey No. 226, containing $10^4$ $TCID_{50}$ of virus, was inoculated intravenously into patas monkey No. 688, an antibody negative animal which previously had never been infected with SHF virus. Titration of serial serum samples from patas monkey No. 688 in rhesus monkey PMAC showed that a persistent infection was established in this animal. Titers of infectious virus in these serial samples ranged between $10^{3.2}$ and $10^{5.2}$ $TCID_{50}$/ml over the 388-day period that the animal was monitored after inoculation. By ELISA, the highest titer of specific IgG antibody to the homologous strain of persistent virus was found to be 10 in serum samples taken from patas monkey No. 688 at 21, 28, 97, 143, 164, 224, and 388 days after infection. Ten (10) is the lowest significant level of antibody above background detectable by our ELISA. Results of this experiment failed to support our postulate that some persistent strains of SHF virus might have the capacity to induce significant levels of specific IgG antibody in infected patas monkeys, while others could not.

Dual infection of patas monkeys, first with an acute strain of SHF virus and then with a persistent strain, could also account for the high titers of antibody detected in some persistently infected animals. In this hypothetical scenario, a patas monkey would undergo an acute infection with a strain of SHF virus such as LVR or P-180, mount an immune response with concomitant synthesis of specific antibody, recover from infection and eliminate the virus. At some later time, this animal could be infected with a persistent strain of virus and develop a chronic infection. This scenario would be possible if the acute and persistent strains of virus were sufficiently different so that the immune response to the acute infection did not abrogate the persistent infection by virus neutralization or other effector mechanisms. Using this rationale, patas monkey No. 327 was infected with LVR strain virus (acute strain). As shown in Table III, patas monkey No. 327 became acutely infected, and mounted a strong antibody response. Virus was no longer detectable in plasma or leukocyte samples from this animal 14 after infection as determined by inoculation of USU-104 cell cultures.

Patas monkey No. 327 was then challenged with P-248 virus (persistent strain) 42 days after it had been inoculated with LVR virus. At this time, the animal had an IgG antibody titer of 6250 to LVR virus antigen and was free of infectious LVR virus. Patas monkey No. 327 became infected with P-248 virus and remained viremic during the 218 days it was monitored (Table III). Thus, a plausible explanation why some persistently infected patas monkeys had high SHF virus antibody titers is that the antibody had been induced by an acute infection from which they had recovered, prior to becoming persistently infected.

*Sensitivity of various cell types to lytic infection with different virus isolates.* The sensitivity of different cell types to lytic infection by the four SHF virus isolates (LVR, P-180, P-248, and P-741) was investigated to provide biological markers to distinguish the individual isolates. The cell types chosen were USU-104 cells, primary rhesus monkey PMAC, and primary patas monkey PMAC. These cell types were chosen because they previously were found to support growth of at least one of the four virus isolates. Pools of the individual virus isolates for infectivity titration were prepared in the following cell types: LVR was grown in USU-104 cells, P-180 was grown in patas monkey PMAC, and P-248 and P-741 were grown in rhesus monkey PMAC. The individual virus pools were diluted in serial 10-fold increments and the dilutions inoculated into USU-104, patas monkey PMAC, and rhesus monkey PMAC. As shown in Table IV, all three cell types were lytically infected by

TABLE III. INFECTION OF PATAS MONKEY No. 327 WITH LVR AND THEN P-248 STRAIN SHF VIRUS

| Infection with LVR virus | | | | Infection with P-248 virus | | |
|---|---|---|---|---|---|---|
| Days after infection | Plasma | Leukocyte associated | IgG[a] antibody titer | Days after infection | Infectivity $Log_{10}$ $TCID_{50}$/ml | IgG antibody titer |
| 0 | <0.7 | <0.7 | <10 | 0 | <0.7 | 6250 |
| 1 | 5.75 | 2.0 | <10 | 13 | 3.7 | N.D. |
| 2 | 5.25 | 1.0 | N.D. | 24 | 3.2 | 6250 |
| 3 | 6.0 | 3.0 | N.D. | 63 | 4.2 | 1250 |
| 4 | 6.25 | 3.25 | 10 | 125 | 4.2 | N.D. |
| 5 | 6.75 | N.D.[b] | N.D. | 218 | 4.2 | 1250 |
| 6 | 6.25 | N.D. | N.D. | | | |
| 7 | 4.25 | 2.75 | 10[c] | | | |
| 10 | 3.0 | <0.7 | 250 | | | |
| 14 | 1.5 | <0.7 | 1250 | | | |
| 21 | <0.7 | <0.7 | 1250 | | | |
| 28 | <0.7 | <0.7 | N.D. | | | |
| 35 | <0.7 | N.D. | 6250 | | | |
| 42 | <0.7 | N.D. | 6250 | | | |

[a] Purified LVR strain SHF virus used as ELISA antigen.
[b] N.D. = not done.
[c] Reciprocal of the serum dilution yielding a positive ELISA result ($\geq 0.2$ $A_{405}$ units above background).

LVR, but only patas and rhesus macrophages were infected by P-180. The P-248 and P-741 isolates lytically infected only the rhesus macrophages. The ability of the individual isolates to lytically infect a particular cell type appears to be a fairly stable characteristic because blind passage of isolates in nonpermissive cell types did not select for mutants which could cause lytic infection. Thus, sensitivity of the specified cell types to lytic infection is a useful marker to distinguish these isolates.

*Do persistent strains of SHF virus replicate in patas monkey PMAC Cultured in Vitro?* We previously reported that the P-248 isolate persistently infected USU-104 cells and replicated for many generations in these cells without causing noticeable cytopathology (7). Patas monkey PMAC cultures were inoculated with P-248 virus to determine whether a similar nonlytic infection might also occur in these cells. To illustrate the growth curve of P-248 virus in a permissive cell type, rhesus monkey PMAC cultures were also infected under the same conditions. As shown in Fig. 1, progeny P-248 virus was detected in rhesus monkey PMAC cultures between 4 and 6 hr after infection. Peak titers of virus were found 10 to 24 hr after infection. The other three isolates (LVR, P-180, and P-741) also had growth curves in rhesus monkey PMAC with a time course of virus production similar to that shown for P-248. In addition, LVR and P-180 had similar growth curves in patas monkey PMAC (data not shown). Conversely, little infectious virus was produced by patas monkey PMAC inoculated with P-248 virus (Fig. 1) or P-741 (data not shown). These results suggest that the strains of SHF virus which produce persistent infections in patas monkeys replicate poorly in *in vitro* cultured PMAC from this species.

TABLE IV. SENSITIVITY OF VARIOUS CELLS TO LYTIC INFECTION BY DIFFERENT SHF VIRUS ISOLATES

| | Cells | | |
|---|---|---|---|
| Virus isolate | USU-104 | Patas monkey PMAC[b] | Rhesus monkey PMAC |
| LVR | 7.7[a] | 7.2 | 8.2 |
| P-180 | <0.7 | 7.2 | 7.2 |
| P-248 | <0.7 | <0.7 | 6.7 |
| P-741 | <0.7 | <0.7 | 6.5 |

[a] Infectivity titer expressed as $Log_{10}$ $TCID_{50}$/ml.
[b] PMAC = *in vitro* cultured peritoneal macrophages.

**Discussion.** Characteristics of the LVR, P-180, P-248, and P-741 isolates are summarized in Table V. Macaque monkeys inoculated with any of the four isolates have developed typically fatal SHF. In fact, all known isolates of SHF virus have produced highly fatal infections in macaque species. In contrast, exper-

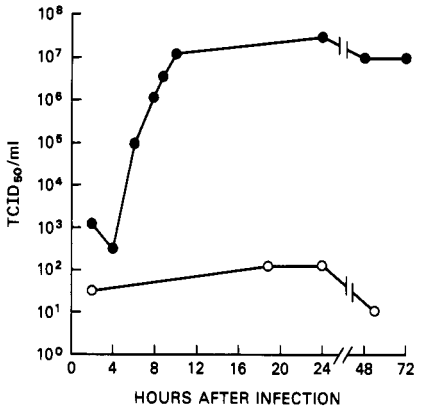


FIG. 1. Replication of P-248 strain SHF virus in rhesus (●) or patas monkey PMAC (○). PMAC cultures in 25-$cm^2$ vessels were infected with about 1 $TCID_{50}$/ml of P-248 virus. After a 1-hr virus adsorption period at 37°C, the cultures were washed three times with EMEM-2 (5 ml/wash) and refed with 5 ml of the same medium. Cultures were incubated at 37°C in 5% $CO_2$ in air and sampled periodically. Titers of infectious virus in samples were determined in rhesus monkey PMAC, a permissive cell type.

imental infections of patas monkeys have only occasionally been fatal. Patas monkeys infected with either LVR or P-180 have developed an acute disease of variable severity, and those inoculated with P-248 or P-741, a long-term, asymptomatic, persistent infection. No exceptions have been noted in the type of infection produced by a particular isolate. Therefore, the type of infection produced by a particular isolate appears to be a rather stable trait of that isolate.

Frequent symptoms seen in acutely infected patas monkeys are fever, anorexia, lethargy, facial edema, dehydration, and in some severe nonfatal cases, small subcutaneous hemorrhages. Generally, these symptoms only occur during the first 10 days of infection, after which the animals usually make a complete recovery. Fatally infected patas monkeys have many of the same clinical and pathological features seen in fatally infected macaques. Apparently, death in patas monkeys also results from a hemorrhagic, hypovolemic shock syndrome.

Of the four isolates studied, P-180 consistently produced the most severe disease in patas monkeys. It also grew to the highest titer in *in vitro* cultures of patas monkey PMAC. P-180 was isolated from the spleen of a patas monkey which died of SHF during a 1972–1973 epizootic at the Corbel Facility, Rockville, Maryland, in which over 100 patas monkeys died. This is the only known instance in which SHF virus has caused high mortality in a captive colony of patas monkeys (W. T.

TABLE V. COMPARISON OF CHARACTERISTICS OF THE LVR, P-180, P-248, AND P-741 ISOLATES OF SHF VIRUS

| Characteristic | Isolate | | | |
|---|---|---|---|---|
| | LVR | P-180 | P-248 | P-741 |
| Physical–chemical structure | Togavirus | Togavirus | Togavirus | Togavirus |
| Type of infection in macaque monkeys | Acute, fatal hemorrhagic disease | Acute, fatal hemorrhagic disease | Acute, fatal hemorrhagic disease | Acute, fatal hemorrhagic disease |
| Type of infection in patas monkeys | Acute disease, usually nonfatal | Severe acute disease, sometimes fatal | Asymptomatic persistent infection | Asymptomatic persistent infection |
| Antigenic relationship | Related | Related | Most distantly related | Related |
| IgG antibody response in patas monkeys | High | High | Low | Low |
| Cytopathology in | | | | |
| USU-104 cells | Lytic | Nonlytic | Nonlytic | Nonlytic |
| Patas PMAC | Lytic | Lytic | Nonlytic | Nonlytic |
| Rhesus PMAC | Lytic | Lytic | Lytic | Lytic |

London, personal communication). All the factors which contributed to this epizootic are not understood, but the heightened virulence of the P-180 strain of virus was probably of major importance.

By ELISA, virions of all four isolates were shown to contain related antigens. Of the four isolates, P-248 was the most distantly related antigenically. Infection of patas monkeys with LVR or P-180 (acute strains) elicited a strong humoral immune response and virus was eventually totally eliminated from the animals. Neutralizing antibody was present in serum from acutely infected animals. However, these sera contained only homeotypic neutralizing activity; i.e., they did not cross-neutralize heterotypic SHF virus isolates. This point can be illustrated by the fact that patas monkey No. 327, an animal which recovered from LVR infection and had an IgG antibody titer of 6250 to the homeotypic virus, could be persistently infected with the P-248 isolate. Regardless of when tested, patas monkeys infected with either of the two persistent strains of SHF virus studied, P-248 or P-741, had only low titers of specific IgG antibodies in their serum and neutralizing activity was never detected in these sera. Neither have we detected virus antibody complexes in sera of persistently infected patas monkeys. The reasons why strains of SHF virus which cause persistent infections in patas monkeys elicit poor humoral immune responses are currently unknown.

We have presented evidence that acute (LVR, P-180) and persistent (P-248, P-741) strains of SHF virus can be identified by their differential ability to lytically infect USU-104, patas monkey PMAC, and rhesus monkey PMAC. LVR could be differentiated from the other three isolates by its unique ability to lytically infect all three cell types. The P-180 isolate could be differentiated from LVR, P-248, and P-741 because it could lyse both patas and rhesus monkey PMAC but not USU-104 cells. The persistent strains, P-248 and P-741, could only lyse rhesus monkey PMAC. All four isolates had the capacity to lytically infect rhesus monkey PMAC, possibly accounting for the extreme sensitivity of rhesus monkeys to fatal SHF.

Although macrophages appear to be the target of infection in macaque monkeys (8), in patas monkeys, the target is less clear. Many different cell types from patas monkeys have been tested *in vitro,* and of these cell types, only adherent cells with monocyte–macrophage characteristics have been found to support substantial replication of acute strains of SHF virus. Both LVR and P-180 lytically infected *in vitro* grown patas PMAC and grew to high titer in these cells. These results suggest that cells of monocyte-macrophage lineage are the targets of acute strains of SHF virus. Conversely, we have presented data showing that persistent strains of SHF virus replicated only minimally, if at all, in *in vitro* cultured patas monkey PMAC. Titers of infectious virus in the serum of patas monkeys persistently infected with the P-248 isolate generally vary between $10^3$ and $10^5$ $TCID_{50}$/ml, whereas the titers of infectious virus in serum of animals infected with the P-741 isolate are even lower. Whether immune or other mechanisms regulate the titers of infectious virus present in persistently infected animals is a perplexing question, since only minimal antibody, without neutralizing activity, is present in the serum of these animals. Currently, it is unknown whether macrophages are the cells which maintain persistent infections. A possibility which we have considered is that macrophages, in only specific stages of differentiation, may be susceptible to infection by persistent strain of SHF virus. These questions will be a focus of future work.

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