Response of Camels to Intradermal Inoculation with Smallpox and Camelpox Viruses

DERRICK BAXBY,* H. RAMYAR, M. HESSAMI, AND B. GHABOOSI

Department of Medical Microbiology, Liverpool University, Liverpool, England*; and Institut d'Etat des Serums et Vaccins Razi, Teheran, Iran

Received for publication 2 December 1974

Recently increasing attention has been paid to poxviruses isolated from animals, particularly to those which share properties with smallpox viruses. Strains of camelpox virus isolated in Iran have an extremely limited host range. Apart from camels (19), the only animals which have been infected successfully are monkeys and infant mice (2); the last two are also susceptible to smallpox viruses (11, 12). Camelpox and smallpox viruses behave similarly in some cell cultures (18), and a detailed comparison of camelpox virus with various smallpox virus strains (2) failed to differentiate camelpox virus from strains of smallpox virus isolated in Tanzania. More recent work has shown that camelpox and smallpox viruses can be differentiated in certain cell cultures (3) and that there are differences in their soluble antigens (10).

In view of the similar experimental host range and laboratory characteristics, it was decided to carry out collaborative experiments in Teheran and Liverpool designed to compare the behavior of camelpox and smallpox viruses in the camel. Such experiments are described here. It was hoped that they would provide information on the relationship between these viruses as well as providing basic information about camelpox, a little-studied disease of some economic importance.

MATERIALS AND METHODS

Virus strains. The Teheran strain of camelpox virus (19) was used after eight passages in lamb kidney cells. An intermediate strain of smallpox virus, EA8 (5), was used after six passages on the chick chorioallantoic membrane (CAM).

Animals. Young camels, approximately 10 months old, were housed in isolation suites of the Razi Institute, Teheran. Virus neutralization and hemagglutination inhibition tests on serum samples taken before infection showed that none of the animals had poxvirus antibody.

Infection experiments. Two animals were inoculated with camelpox and two with smallpox virus. The animals were kept in strict isolation and the smallpox infection experiment was completed before the start of the camelpox experiment.

Virus was inoculated intradermally into the previously cleaned and shaved thoracic sides. Three inoculations of 0.3 ml per dilution were used. Tenfold dilutions were used; for camelpox the virus doses were $1.8 \times 10^4$ to $1.8 \times 10^{-1}$ mean tissue culture infective doses (TCID₅₀) (titrated in lamb kidney cells); for smallpox the doses were $3 \times 10^8$ to 3 pox-forming units (PFU) (titrated on the CAM).

The animals were examined daily, and 3, 5, 7, 9, 11, and 21 days after infection blood samples were taken for virus isolation and serum samples were taken for antibody studies.

Forty-one days after inoculation with smallpox virus, the animals were challenged by intradermal inoculation of $6 \times 10^6$ TCID₅₀ of camelpox virus, and a final serum sample was taken after 72 days. The animals originally inoculated with camelpox virus were challenged after 28 days with $10^7$ PFU of smallpox virus, and the final serum sample was taken on day 35.

Two control animals were included in the animal room at the time that the camelpox experiment was started. They were examined daily, crusts were taken
for virus isolation, and the animals were bled to provide serum samples 35 days after exposure.

**Virus isolation.** In Teheran, crusts from lesions and whole-blood specimens were inoculated into cultures of primary lamb kidney and Vero cells. In Liverpool, whole blood and separated leukocytes (4) were inoculated into HeLa cells and onto the CAM. In all cases negative cultures were passaged through two further subcultures.

**Serological tests.** Serum specimens were tested in Liverpool. All specimens were inactivated at 56°C for 20 min before the test.

Complement-fixing antibody was titrated by using 3 MHD of preserved guinea pig complement, 3 units of antigen, and overnight fixation at 4°C.

Hemagglutination (HA)-inhibiting (HAI) antibody was titrated by reacting 4 HA units with serum dilutions at 35°C for 1 h before adding 1% fowl erythrocytes suspended in 1% normal rabbit serum.

Antigens for the above tests were the supernatant from high-speed (10,000 x g) centrifugation of virus-infected CAM.

Virus neutralization (VN) tests were done by reacting serum dilutions with virus at 35°C for 2 h, at which time residual virus was detected by CAM inoculation. The virus suspensions (intracellular virus) used for this test were artificially extracted from the CAM and would not detect antibody directed against the additional antigens possessed by naturally released (extracellular) virus (6). Antibody to extracellular virus was detected by its ability to inhibit secondary plaque and "comet tail" formation when added to the liquid medium overlaying infected HeLa cell monolayers (6).

**Vaccinia infection of rabbits.** To provide a suitable basis for comparison of the serological response in the infected camels, a series of serum specimens was obtained from rabbits bled at various time intervals after a single intradermal inoculation of 3 x 10^6 PFU of vaccinia virus.

In all cases the serum titer given is based on the serum dilution before addition of virus, antigen, etc.

**RESULTS**

**Response to intradermal inoculation.**

(i) Camelpox. Camelpox virus was infective for camels by the intradermal route at the lowest dose tested (1.8 x 10^-1 TCID₀). Papules started to develop after 5 days and progressed through pustules and vesicles by 6 to 8 days. Crusts began to form by 9 to 10 days.

Secondary, generalized lesions appeared on camel A in 9 days and on camel B in 11 days. The progression of the lesions from papules to crusts followed the same time sequence as the primary lesions. Both animals had recovered by 4 weeks.

Virus was isolated from the primary and secondary lesions and also on day 7 from the blood of both animals.

The results described above are essentially what one would expect from intradermal inoculation of a relatively pathogenic poxvirus into its natural host.

(ii) Smallpox. In contrast to the above, intradermal inoculation of camels with smallpox virus resulted in only slight visible effect. The highest dose tested (3 x 10^6 PFU) produced only a small inflamed swelling which disappeared within a week. Attempts to isolate virus from such swellings and from blood samples were unsuccessful.

These results, besides indicating quite marked differences in the pathogenicity of these viruses for the camel, suggest that the camel need not be considered as a potential host or reservoir for smallpox virus.

**Response to cross-challenge.** In view of the very slight effect of smallpox virus in camels, it was not surprising that the two animals initially infected with camelpox virus resisted challenge with smallpox virus.

However, both the camels which had initially been inoculated with smallpox virus resisted challenge with a dose of camelpox virus which produced generalized infection in fully susceptible animals. This result, besides confirming in vivo the immunological relationship between the viruses demonstrated earlier in vitro (2), also suggested that inoculation of the camel with smallpox virus was sufficient to stimulate a considerable immune response.

**Serological response to camel and smallpox viruses.** (i) Camelpox. Figure 1 shows the development of VN and HAI antibody to camelpox virus. Antibody first became detectable by 7 to 9 days after infection and reached a peak by 11 days or shortly thereafter.

Maximum levels of VN antibody were 1:8,000 for camel A and 1:3,000 for camel B. Maximum levels of HAI antibody were 1:8,000 for camel A and 1:3,000 for camel B. This was similar to the response of the rabbit to intradermal infection with vaccinia virus. In this case, VN and HAI antibody was detected at 7 to 9 days and reached peak titers of 1:5,000 (VN) and 1:1,500 (HAI) by day 15.

Neither of the camels inoculated with camelpox virus developed a rise in antibody levels after challenge with smallpox virus. This was probably due to the high level of circulating antibody already present which would eliminate the challenge antigen.

(ii) Smallpox. Figure 2 shows the development of VN and HAI antibody to smallpox virus. In general, antibody was first detected later and reached lower maximum titers than in the animals infected with camelpox virus. The maximum antibody levels detected before challenge were 1:170 (VN) and 1:200...
(HAI) in camel C, and 1:80 (VN) and 1:50 (HAI) in camel D.

Sera from the smallpox-infected camels prevented secondary plaque formation by virus when used in overlay medium at dilutions of 1:150 (camel C, day 21) and 1:50 (camel D, day 21). This indicated that limited virus replication had taken place (see Discussion).

After challenge with camelpox virus, there was a boost in antibody levels in camel D but not in camel C, perhaps indicating differences in the degree of immunity induced by the initial infection.

Complement fixation. All serum samples were tested for complement-fixing antibody. However, many of the samples showed anti-complementary activity. The few unequivocal results obtained indicated that complement-fixing antibody was first detected on days 9 to 11 and reached a peak level of about 1:500 by day 21.

Natural transmission of camelpox. Two control camels were exposed to the camelpox-infected animals at the time the latter were inoculated. The two contact animals developed generalized infection 13 days after exposure. The lesions progressed through their normal course and the animals recovered within 4 weeks. Serum samples taken 35 days after exposure had VN titers of 1:2,000 and 1:3,000 and HAI titers of 1:2,000 and 1:2,000.

DISCUSSION

Camelpox virus is of interest not only as a pathogen of its natural host but also because of its similarity to smallpox virus in a number of standard tests used for the laboratory diagnosis of smallpox (2, 3). Consequently the behavior of smallpox virus in camels is also of interest.

The results of the present experiments have shown marked differences in the behavior of smallpox and camelpox viruses in camels. They reinforce the differences reported recently in their behavior in certain cell cultures (3) and in gel diffusion tests (10). Although an unnatural route of infection and laboratory-passaged virus was used, the experiments confirm epidemiological evidence that camels are unlikely to act as alternative hosts for smallpox virus.

It is perhaps of interest to discuss whether limited replication of smallpox virus took place in the camel or whether the virus was acting as a nonreplicating, i.e., noninfectious, antigen. The lesions at the inoculation site were insignificant and virus was not recovered from them. However, the animals resisted completely a severe challenge with camelpox virus, and noninfec-
tious poxvirus vaccines frequently fail to protect against challenge (6).

If virus suspensions are freed from HA before inoculation into animals, then development of antibody to HA can be taken as evidence of virus replication in the host concerned (13, 22). On the other hand, large doses of noninfectious virus contaminated with HA will elicit HAI antibody (16). Smallpox virus suspensions, in particular, prepared from infected CAM have low HA titers initially (K. McCarthy, M.D. thesis, Liverpool Univ., 1951), and the methods used here to prepare the virus inoculum removed all detectable HA activity (Baxby, unpublished data). Therefore, the development of HAI antibody in camels inoculated with smallpox virus suggests that virus replication occurred.

Additional evidence that smallpox replication had taken place is provided by the fact that the camels developed antibody which prevented secondary plaque formation in tissue culture, i.e., which neutralized naturally released extracellular virus. Antibody directed against the surface components of artificially released intracellular poxvirus will not neutralize extracellular virus (1, 6, 21). The virus used to inoculate the camels was prepared from infected CAM and purified by differential high-speed centrifugation and Arcton treatment and dispersed by ultrasonic treatment. This damages the outer coat of extracellular but not intracellular virus (6; G. Appleyard and E. A. Boulter, personal communication) and reduces the amount of extracellular virus in the smallpox inoculum to <0.1% (Baxby, unpublished data). Noninfectious extracellular virus is poorly antigenic (1, 6, 21), and had the inoculum been acting as a noninfectious antigen, we would not have expected antibody to extracellular virus to develop. Therefore, the most likely interpretation of the resistance to challenge and development of antibody to HA and to extracellular virus is that limited virus replication took place in the camel.

We have obtained limited data about the pathogenesis of camelpox, namely, incubation period, development of lesions, and ease of natural transmission. This information is entirely consistent with what one would expect of a poxvirus producing a generalized infection in its natural host as discussed previously for rabbitpox (4), smallpox (9), and monkeypox (7). It should be noted that infection was initiated with 1.8 \times 10^{-1} TCID_{50} as titrated in lamb kidney cells. This indicates that these cells are of lower sensitivity to camelpox virus than to intradermal inoculation of the camel itself.

Information is still required about the epidemiology and natural history of poxvirus infections in camels, particularly with a view to assessing the distribution and relative importance of the various viruses involved. The virus strains studied by us cause camelpox in Iran, and similar if not identical viruses have been isolated from outbreaks in the U.S.S.R. (17) and the Yemen (Baxby and Barrow, unpublished data). However, poxvirus infections of camels have also been caused by vaccinia virus (14) and by a virus resembling Orf (20).

Due to the very close relationship between smallpox and camelpox viruses, there may be some confusion over laboratory identification. The slight serological differences detected by gel diffusion techniques are at present best demonstrated by using specifically absorbed reagents (10), and immunological identity appears complete (2). The viruses can most easily be differentiated by correct choice of cell cultures (3). Serious repercussions from incorrect identification could result if human camelpox was more common. Although there are some references to human camelpox (8, 15), recent inquiries suggest that it is relatively rare (Ramyar, unpublished data). This could have been brought about in part by the high degree of immunity induced via the World Health Organization Smallpox Eradication Campaign. Whether human camelpox will become more common as the immunity of the human population wanes will be a subject for long-term inquiries.

**LITERATURE CITED**


