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Antigenic Variation of Caprine Arthritis–Encephalitis Virus during Persistent Infection of Goats

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SUMMARY

Six caprine arthritis–encephalitis virus (CAEV)-free goats kept in strict isolation were inoculated intravenously with a cloned CAEV isolate (virus 020). At 78 weeks post-infection a virus (virus 095) isolated from one of the goats was shown to have the characteristics of CAEV, but was antigenically distinct from virus 020 and two other CAEV isolates by serum neutralization tests. Serum from the goat that had the variant virus neutralized the inoculum virus and the variant virus but serum from other inoculated goats neutralized only the inoculum virus. The variant virus and the inoculum virus were shown to co-exist in the infected goat, but the presence of the antigenic variant did not appear to be associated with an increase in severity of lesions compared with other inoculated goats.

INTRODUCTION

Antigenic variation is one mechanism micro-organisms use to avoid the immune response of the host (Porter, 1975). This phenomenon is well known with several virus types, including influenza virus (Laver & Webster, 1968), foot-and-mouth disease virus (Hyslop & Fagg, 1965) and lentiviruses such as equine infectious anaemia virus (Kono et al., 1973; Crawford et al., 1978) and maedi–visna virus (Petursson et al., 1976; Narayan et al., 1977a, b).

Antigenic variation may be one explanation for the production of persistent virus infections in animals. Antigenically novel mutants which arise in an infected animal may not be neutralized by pre-existing antibody and can replicate in preference to the original infecting strain of virus. Serum from Icelandic sheep persistently infected with visna virus neutralized the inoculum strain of virus, but not the virus isolated at the time of collection of serum (Gudnadottir, 1974). Similar findings were subsequently reported in infected Border Leicester and Hampshire sheep (Narayan et al., 1977a, b, 1978). The antigenic variation in visna virus was shown to result from oligonucleotide changes in the 3'-terminal region of the viral genome (Clements et al., 1982) which is the region that codes for the envelope glycoproteins of the virus. These proteins have been shown to be at least one of the antigenic targets for neutralization of virus (Scott et al., 1979).

Antigenic variation between different caprine arthritis–encephalitis virus (CAEV) strains has been previously reported by Narayan et al. (1984) and Ellis et al. (1987). Neutralizing antibody induced by hyperimmunization of goats with CAEV mixed with inactivated Mycobacterium tuberculosis adjuvant (Narayan et al., 1984) neutralized the homologous strain of CAEV but not other CAEV strains. Ellis et al. (1987) also detected antigenic variation between CAEV isolates using antiserum from naturally infected goats. There are, however, no reports of antigenic variation of CAEV occurring within a persistently infected goat. This paper reports, apparently for the first time, the demonstration of antigenic variation of CAEV during persistent infection of a goat.
METHODS

Viruses. CAEV strains used included virus 020 which was originally isolated from lung explant cultures from a British Alpine nanny with generalized interstitial pneumonia, virus 151 from synovial membrane explant cultures from a Saanen nanny with arthritis-synovitis and virus 87 from lung explant cultures from an Anglo-Nubian nanny with generalized interstitial pneumonia. Virus 151 had been cloned by plaque purification as described previously (Ellis et al., 1983) and viruses 020 and 87 were cloned by a series of three terminal dilutions in goat synovial membrane (GSM) cell cultures. Tenfold dilutions of virus were inoculated into five wells of a microtitre tray containing confluent GSM cell monolayers and incubated at 37 °C in a 5% CO₂-in-air atmosphere. Wells in the microtitre trays at the highest dilution of virus that contained a single syncytium were harvested 14 days post-infection (p.i.) by freezing and thawing. The terminal dilution was then repeated twice more. The cloned viruses were shown to have cultural, morphological, physicochemical and antigenic properties characteristic of CAEV (Ellis, 1986).

Virus titration. The TCID₅₀ was determined by inoculating tenfold dilutions of virus suspension into five wells of a microtitre tray containing 80 to 90% confluent GSM cell monolayers. The cultures were incubated at 37 °C in a 5% CO₂-in-air atmosphere for 14 days and then examined for c.p.e. The TCID₅₀ was calculated by the method of Reed & Muench (1938).

Experimental infection of goats and re-isolation of virus after infection. Six Angora-cross, CAEV-free, castrated male goats (090, 091, 093, 094, 095 and 096) were isolated in a completely enclosed 20 m² room that had separate positive pressure ventilation, water supply and drainage. Entry to the room was via an ante-room where food was stored and entry to this area was through two footbaths containing disinfectant solution (Stericide®, ICI). The goats were inoculated intra-articularly and intravenously with 9 × 10⁶ TCID₅₀ of CAEV isolate virus 020.

At intervals after infection isolation of virus from peripheral blood leukocytes was attempted from the infected goats. Virus isolation involved co-cultivation of peripheral blood leukocytes from citrate-treated blood samples with GSM cell cultures as described previously (Ellis et al., 1983). When c.p.e. was apparent, the co-cultivated cells were frozen and thawed twice, clarified by low speed centrifugation and re-inoculated onto other GSM cells. These infected cultures were harvested when the c.p.e. appeared maximal and were used as stock virus preparations for further study. Serum samples from the six infected goats were collected at the times shown in Table 4. These were examined for neutralizing antibody against viruses re-isolated from the infected goats, virus 020 and for some goats viruses 151 and 87 using a constant virus, varying serum neutralization test.

Serological tests. Two neutralization test procedures were used. A constant virus, varying serum neutralization test similar to that described for ovine progressive pneumonia virus by Narayan et al. (1978) was used for serological examinations of infected goats. Twofold dilutions of serum that had been heated at 56 °C for 30 min were made in GSM cell growth medium. These dilutions were mixed with an equal volume of CAEV containing 10⁶ TCID₅₀/0.1 ml and incubated at 37 °C for 45 min. Each dilution mixture was inoculated into four replicate cultures of GSM cells in microtitre plates and incubated at 37 °C in a 5% CO₂-in-air atmosphere. Serum neutralization tests were read at 10 to 14 days p.i. and neutralization titres were expressed as the reciprocal of the highest dilution that prevented viral c.p.e. in GSM cells.

A constant serum, varying virus neutralization was used to identify clones of the variant virus. Tenfold dilutions of the re-isolated viruses and virus 020 were made in GSM cell culture growth medium. To each dilution was added an equal volume of a 1:10 dilution of serum collected from the infected goats 82 weeks p.i., or growth medium. The serum was heated at 56 °C for 30 min prior to use. The mixtures were incubated at 37 °C for 45 min and the TCID₅₀ was determined as described above. A neutralization index for each of the goat antisera was determined by subtraction of the TCID₅₀ of the virus with antiserum from the titre without antiserum. The neutralization index of pre-inoculation serum from goats 090 and 095 was also determined.

To minimize the amount of non-infectious virus particles in the serum neutralization test virus stocks, the stock solutions of the various virus isolates used in the neutralization tests were prepared by harvesting the medium from infected GSM cell cultures at the time when viral titres first reached their peak. Agarose gel immunodiffusion (AGID) test antigens prepared from virus 020 and the re-isolated viruses by 20-fold concentration and ether treatment of pooled weekly harvests of tissue culture supernatants from infected GSM cell cultures were compared in AGID tests using procedures described by Cockley et al. (1984).

Cloning and characterization of re-isolated viruses. Re-isolated viruses were cloned by a series of three terminal dilutions in GSM cells using the same procedure described above. Neutralization of the cloned viruses was then examined using constant serum, varying virus neutralization tests against goat serum from various sources.

The cultural and growth characteristics of a virus clone which appeared to be antigenically different from the inoculum virus was compared to the original virus inoculated into the goats. The viruses were inoculated onto monolayers of GSM cells at an m.o.i. of 10. After adsorption for 1 h the monolayers were washed five times with phosphate-buffered saline, growth medium was added and the cultures incubated at 37 °C in a 5% CO₂-in-air atmosphere. The cultures were examined for the presence of syncytia and for virus infectivity at intervals after infection. The electron microscopical characteristics of the viruses in infected GSM cells were examined using the technique described previously (Ellis et al., 1983).
Table 1. Serum neutralization titres* against viruses 020, 090 and 095 for Angora-cross goats inoculated with virus 020

<table>
<thead>
<tr>
<th>Source of serum</th>
<th>Time of collection (weeks p.i.)</th>
<th>CAEV isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>Virus 020</td>
<td>Virus 090</td>
</tr>
<tr>
<td>090</td>
<td>82</td>
<td>64</td>
</tr>
<tr>
<td>091</td>
<td>82</td>
<td>16</td>
</tr>
<tr>
<td>093</td>
<td>82</td>
<td>64</td>
</tr>
<tr>
<td>094</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>095</td>
<td>82</td>
<td>32</td>
</tr>
<tr>
<td>096</td>
<td>48</td>
<td>8</td>
</tr>
</tbody>
</table>

* Titres less than 4 are recorded as negative (−).

Antiserum. Goat anti-CAEV serum was obtained from the following sources. (i) The six goats inoculated with virus 020 at the times p.i. shown in Table 1. (ii) Serum from a CAEV-free goat (373) 12 months after it had been experimentally infected with CAEV strain virus 151 and had neutralizing antibody to that virus. (iii) Serum from a CAEV-free goat (093) 18 months after it had been experimentally infected with CAEV strain virus 020 and had neutralizing antibody to that virus. (iv) Serum from a naturally CAEV-infected goat (33) that was found to have a relatively high monospecific serum neutralization titre to CAEV strain, virus 87.

One goat (095), from which a virus was isolated that appeared antigenically different to the inoculum virus, was hyperimmunized with the re-isolated antigenically different isolate. The cloned re-isolated virus was homogenized with a killed M. tuberculosis/mineral oil adjuvant and the goat was given a series of inoculations commencing at 147 weeks p.i. using the protocol described by Narayan et al. (1984). Serum was collected from the goat 11 weeks after commencing the hyperimmunization.

RESULTS

Isolation of viruses from inoculated goats

Virus was isolated from peripheral blood leukocytes from two goats, 090 and 095 (designated as isolates 090 and 095 respectively), at 78 weeks p.i. and again from goat 095 (designated isolate 095-1) at 104 weeks p.i. Isolated syncytia were first observed in the GSM cell monolayers of the co-cultures 10 to 14 days after starting the cultures. After a single passage in GSM cells, similar titres of $10^{4.67}$, $10^{4.33}$ and $10^{5.5}$ TCID$_{50}$/0.1 ml for virus isolates 090, 095 and 095-1 respectively were detected 10 days p.i.

Prior to cloning of virus isolates 090 and 095, the neutralization titres to those viruses and virus 020 in serum from the six goats inoculated with virus 020 were determined and are shown in Table 1. Identical neutralization titres to virus 020 and virus isolate 090 were detected in all goats suggesting that these two viruses were antigenically similar. Neutralizing antibody to virus isolate 095 was detected only in goats 090, 093 and 095. In the serum of goats 090 and 093, the neutralization titres to virus isolate 095 were eight- to 16-fold less than against the other two virus isolates examined, suggesting virus isolate 095 was antigenically different to the original virus 020 inoculated into the goats. The neutralization indices of serum from goat 090 against viruses 095-1 and 020 were 2-3 and 3-0 respectively and for serum from goat 095 against the same viruses were 2-0 and 2-3 respectively, suggesting that virus isolate 095-1 was antigenically similar to virus 020.

The neutralization indices of four clones of virus 095 using serum from goats 090 and 095 are shown in Table 2. The neutralization index of serum against clone A was similar to that for virus 020, suggesting that this clone was antigenically indistinguishable from virus 020. However, clones B, C and D were neutralized by serum from goat 095, but not goat 090, suggesting these three clones were antigenically distinct from virus 020 which was used to infect the goats. Clone D (designated as virus isolate 095 D) was used for all further serum neutralization tests and for characterization studies of virus isolate 095.
Table 2. *Neutralization indices of cloned virus 095 suspensions using serum from goats 090 and 095*<sup>+</sup>

<table>
<thead>
<tr>
<th>Virus</th>
<th>Clone</th>
<th>Serum from goat</th>
<th>Neutralization index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>095</td>
<td>A</td>
<td>090</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>095</td>
<td>2.3</td>
</tr>
<tr>
<td>095</td>
<td>B</td>
<td>090</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>095</td>
<td>2.3</td>
</tr>
<tr>
<td>095</td>
<td>C</td>
<td>090</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>095</td>
<td>2.3</td>
</tr>
<tr>
<td>020</td>
<td></td>
<td>090</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>095</td>
<td>2.3</td>
</tr>
</tbody>
</table>

* The serum from goats 090 and 095 used in these studies had been collected at 82 weeks p.i. with virus 020.
† The neutralization index was determined by subtracting the TCID<sub>50</sub> of the virus/antiserum mixture from the TCID<sub>50</sub> of the virus without antiserum. Incubation of the virus suspension with a 1:10 dilution of pre-inoculation serum from goats 090 and 095 did not result in a reduction in virus titre.

Table 3. *Serum neutralization titres against various CAEV isolates in goats experimentally infected with virus 020*

<table>
<thead>
<tr>
<th>Goat no.</th>
<th>Virus</th>
<th>Time post-inoculation (weeks)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>095*</td>
<td>020</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>095</td>
<td>†</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td></td>
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<tr>
<td></td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>090</td>
<td>020</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>095</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>151</td>
<td></td>
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<tr>
<td>091</td>
<td>020</td>
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<td></td>
<td>095</td>
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<tr>
<td>093</td>
<td>020</td>
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<td>094</td>
<td>-</td>
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<tr>
<td></td>
<td>095</td>
<td>-</td>
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<tr>
<td>096</td>
<td>020</td>
<td>-</td>
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<tr>
<td></td>
<td>095</td>
<td></td>
</tr>
</tbody>
</table>

* Goat 095 was hyperimmunized with virus 095 plus killed *M. tuberculosis*/mineral oil adjuvant commencing 147 weeks p.i.
† - Titre less than 4.
‡ Sacrificed at or prior to this time.

*Characteristics of replication of virus isolate 095 D in cell culture*

When inoculated onto GSM cell monolayers, virus isolate 095 D caused a c.p.e. characterized by syncytium formation which was indistinguishable from that observed in other CAEV isolates. When virus isolate 095 D was inoculated onto GSM cell monolayers at an m.o.i. of 10, cell fusion was first observed 8 h p.i., which was 12 h before infectious virus was first detected in the cultures. Peak viral titres were reached at 120 h p.i.

Transmission electron microscopical examination of GSM cells infected with virus isolate 095 D showed spherical virus particles 80 to 110 nm diameter on the cytoplasmic membranes of vacuolated cells and pleomorphic C-type budding of virus from cytoplasmic membranes.
Antigenic variation of CAEV

Fig. 1. Precipitin lines of identity formed between serum from goat 090 (wells 1 and 4) and antigens prepared from virus 020 (centre well) and virus 095 D (wells 2 and 5). Goat 090 was experimentally infected with virus 020. Wells 3 and 6 contain phosphate-buffered saline.

Table 4. Results of antigenic comparisons between viruses 151, 020, 87 and 095 using reciprocal serum neutralization tests

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Goat no.</th>
<th>Virus inoculated</th>
<th>Time of collection (weeks p.i.)</th>
<th>CAEV isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>151 020 87   095</td>
</tr>
<tr>
<td>373</td>
<td>151</td>
<td>52</td>
<td></td>
<td>20*</td>
</tr>
<tr>
<td>093</td>
<td>020</td>
<td>74</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
| 33        |^
| 095       | 095‡     | 158              |                     | 10 40 40 20  |

* Values given are serum neutralization titres. Values less than 5 are recorded as negative (−).

† Dairy goat 33 was naturally infected with CAEV but was found to have a high serum neutralization test titre to virus 87.

‡ Serum from goat 095 was collected after infection with virus 020 and a hyperimmunization course with cloned virus 095 plus killed *M. tuberculosis* adjuvant as described by Narayan *et al.* (1984).

Detection of neutralizing antibody to CAEV isolates in inoculated goats

The serum neutralization titres against virus 020 and virus isolate 095 D in serum collected from the six goats infected with virus 020 are shown in Table 3. Serum neutralization titres against virus strains 151 and 87 in sera from goats 090 and 095 are also shown in Table 3. Prior to 80 weeks p.i., neutralizing activity was detected only against the inoculum virus strain 020. The time between infection and the first detection of neutralizing antibody to virus 020 was 22 weeks (± S.D. 7 weeks). Neutralizing activity against virus isolate 095 D was first detected at 80 weeks p.i. in goats 095, 090 and 093, concurrent with an increase in titre to virus 020. Neutralizing activity against both virus 020 and virus 095 was consistently present after 80 weeks p.i. Neutralizing antibody to viruses 87 and 151 was also detected after 80 weeks p.i. in goat 095 and after 132 weeks p.i. in goat 090.

Antigenic relationships between virus isolate 095 D and other CAEV isolates

In an AGID test the precipitin line formed between serum from goat 090 and an antigen prepared from virus 020 showed complete identity with the precipitin line formed between the serum and a similarly prepared antigen from virus isolate 095 D (Fig. 1).

The results of reciprocal serum neutralization tests against viruses 151, 020, 87 and virus isolate 095 D are shown in Table 4. Serum from goats 373, 093 and 33 neutralized
monospecifically viruses 151, 020 and 87 respectively. None of these sera had detectable neutralizing activity against virus isolate 095 D. Serum obtained after hyperimmunization of goat 095 with virus isolate 095 D neutralized all four CAEV isolates.

DISCUSSION

A virus (095 D) isolated from the peripheral blood leukocytes of a goat (095) 78 weeks after inoculation with cloned CAEV 020 was shown to have characteristics which were typical of CAEV isolates previously examined but was not neutralized by serum that neutralized virus 020. Cloned virus 095 D was shown to cause a syncytial c.p.e. and have similar kinetics of replication and cell fusion to the inoculum virus, and to other CAEV isolates described previously (Ellis et al., 1985; Ellis, 1986). The virus formed by C-type budding from cytoplasmic membranes and had the same dimensions and morphology as reported for other isolates of CAEV (Narayan et al., 1980; Crawford et al., 1980; Dahlberg et al., 1981). Additionally, a common antigen in virus 095 D and virus 020 was demonstrated by the use of AGID tests. Prior to isolation of virus 095 D, serum from all six goats neutralized the inoculum virus (020) but not virus 095 D. However, at 80 weeks p.i. serum from goat 095 neutralized both virus 020 and 095 D. This suggested that the virus isolate 095 D was an antigenic variant of virus 020 used to infect the goats, and may have arisen by mutation during the course of the persistent infection induced in the goats. Similar antigenic variants have been reported to occur following infection of sheep with maedi–visna virus (Gudnadottir, 1974; Narayan et al., 1978).

It is considered that virus 095 D was a variant of virus 020 and not an introduced or a contaminant virus present in the original inoculum for the following reasons. First, virus 020 used to infect the goats had been cloned three times. Second, the six goats inoculated with this virus were kept in strict isolation. Third, the highest levels of antibody against virus isolate 095 D were detected in the goat from which the virus was isolated, and no antibody to this virus was detected in any of the six goats until 80 weeks p.i. Fourth, no antigenic cross-reactivity was demonstrated between the cloned virus isolate 095 D, the inoculated virus (020) and other CAEV isolates (Table 4). These were the only CAEV strains which were used in the laboratory while the goat inoculation studies were in progress.

Antibodies to the antigenically variant virus 095 D were detected at 80 but not at 72 weeks p.i. Assuming that the time between inoculation and first appearance of neutralizing antibody against virus 020 (mean 22 weeks ± s.d. 7 weeks) was similar for the production of antibodies to the virus isolate 095 D, it is likely that the earliest time the variant could have developed was between 36 and 44 weeks p.i. These time intervals are similar to those reported in visna virus-infected sheep, where neutralizing antibodies are often not detectable until 3 to 5 months p.i. (Georgsson et al., 1980; Sihvonen, 1981) and antigenic variants of visna virus in inoculated sheep have not been detected until 10 months p.i. (Narayan et al., 1978).

From the co-cultures of peripheral blood leukocytes derived from goat 095 at 78 weeks p.i., two antigenic types of CAEV were obtained during the cloning procedures. One type was indistinguishable from the inoculum virus 020 in neutralization tests and the other, which was designated virus 095 D, did not cross-react with virus 020. This indicated that the original inoculated virus and the antigenic variant were simultaneously present in the peripheral blood leukocytes of the goat, and that the original virus had not been replaced by the variant. A virus of the same antigenic type as the inoculum virus 020 was also isolated 26 weeks later at 104 weeks p.i. A similar observation that antigenic variants persist together with the inoculated strain of virus has been reported previously in sheep persistently infected with visna virus (Narayan et al., 1978; Lutley et al., 1983). During persistent visna virus infection in sheep the antigenic change occurs continuously during the course of the infection (Narayan et al., 1977a, b, 1978; Nathanson et al., 1985).

In visna virus-infected sheep there is also a progressive broadening of the neutralizing antibody spectrum against other visna virus strains, similar in extent to that occurring in sheep hyperimmunized with visna virus (Narayan et al., 1978, 1981). Broadening of the neutralizing antibody spectrum, related to the appearance of antibody to virus isolate 095 D, was also detected in the goats inoculated with virus 020. Soon after virus 095 D was isolated, the goat
from which the virus was isolated had developed neutralizing antibody against the homologous virus, an increase in neutralizing antibody titre to the inoculum virus, and neutralizing antibody to virus 151 was also detected. Eighteen weeks later, neutralizing activity against the antigenetically different virus 87 (Ellis et al., 1987) was also detected. A similar broad neutralizing antibody spectrum was subsequently observed in goat 090 from 132 weeks p.i. onwards.

The biological role of antigenic variation of visna virus in persistently infected sheep has not been established (Nathanson et al., 1985). Although antigenic shift of the virus has been proposed as a mechanism of virus persistence (Narayan et al., 1977a, b), if antigenic variation were important for the persistence of visna virus infection the frequency of isolation of variants in persistently infected sheep should increase (Nathanson et al., 1985). In practice this has not been observed (Narayan et al., 1978; Lutley et al., 1983) and antigenic variation does not appear to be essential for virus persistence (Nathanson et al., 1985). The sequential replication of a series of variant visna viruses in the central nervous system has also been proposed as one potential mechanism for the progressive neurological disease in infected sheep (Narayan et al., 1978). However, Lutley et al. (1983) were unable to demonstrate antigenic variation of visna virus isolates from the central nervous system of sheep with clinical visna. Similarly, in the CAEV-infected goat from which the antigenically variant virus 095 D was isolated, there was no apparent role for the antigenic variant in the persistence of the virus infection because the inoculum strain of virus was still present 26 weeks later. The antigenic variants did not appear to be associated with an increase in severity of the lesions induced in goats; the severity of the joint lesions in the goat from which the variant virus was isolated was similar to that in the other goats infected with the same virus (Ellis, 1986).

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