Slow Virus Replication: the Role of Macrophages in the Persistence and Expression of Visna Viruses of Sheep and Goats

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SUMMARY

Lentiviruses of sheep and goats cause slowly progressive diseases of the central nervous system (visna), lungs (maedi) and joints (arthritis) in their natural hosts. However, the virus target cell(s) in these diseases are still unknown. In this report, using laboratory-adapted Icelandic visna virus and several field strains recently obtained from sheep and goats with natural disease in the U.S.A., we show that macrophages became persistently infected when inoculated in culture. Furthermore, macrophages were an invariable source of virus from experimentally and naturally infected animals. Virus-producing macrophages developed minimal cytopathic changes and virus assembly occurred mainly intracellularly, accumulating in cytoplasmic vacuoles. In contrast to macrophages, sheep choroid plexus fibroblasts developed syncytial cytopathic changes after inoculation and virus maturation occurred at the cell surfaces. Replication of the Icelandic virus was highly productive in this system but that of the field viruses was very inefficient. In some cases these agents failed to replicate in the fibroblasts and no cytopathic effect occurred. This block in the field virus replication was, however, overcome when infected non-producer fibroblasts were co-cultivated with macrophages. In these cases, virus production with attendant cytopathic effect in the fibroblasts required the continuous presence of macrophages because the cells reverted to a non-productive state when separated from macrophages and became productive again when subcultures were added to new macrophages. The roles of the macrophage as a virus target cell and virus inducer in the virus–macrophage–fibroblast interactions are discussed with inferences to the well-known phenomenon of restricted virus replication in infected animals and the immunopathological aspects of the diseases.

INTRODUCTION

Visna and maedi are slowly progressive encephalitic and pneumonic diseases of sheep caused by retroviruses (Thormar & Palsson, 1967; Lin & Thormar, 1970). The disease complex was first described in Iceland where it has since been eradicated by slaughter of affected sheep (Sigurdardottir & Thormar, 1964). Viruses from Icelandic cases have been maintained in the laboratory and subpassaged multiple times through sheep and sheep cell cultures. These laboratory-adapted strains of visna virus have been used for studies of pathogenesis of the disease (Petursson et al., 1976; Griffin et al., 1978), antigenic drift (Narayan et al., 1978, 1981; Clements et al., 1980a) and in vitro replication (Haase & Varmus, 1973; Brahic & Haase, 1978; Clements et al., 1979). Recently, visna-maedi disease
in sheep has been recognized in many parts of the world (De Boer, 1975; Cutlip & Laird, 1976; Dukes et al., 1979; Dawson, 1980; Sheffield et al., 1980) and a visna-like encephalitis–arthritis complex in goats (Cork et al., 1974) was identified on a farm in the United States. A few of these viruses have been isolated and shown to share antigenic determinants with the Icelandic visna-maedi viruses (Narayan et al., 1980; Clements et al., 1980b; Dahlberg et al., 1981).

Tissue culture-adapted Icelandic visna viruses grow to high titre in fibroblastic cells derived from the choroid plexus of sheep. The replication cycle is about 24 h and replication occurs by means of a proviral DNA intermediate which integrates into the host cell DNA (Haase & Varmus, 1973). Numerous copies of virus RNA are transcribed from this template and virus maturation occurs at the cell membrane (Coward et al., 1970). Cytopathic effects consist of multinucleated giant cell formation (fusion) which coincides with the period of peak virus production. Cell fusion by the virus can, however, occur independently of virus replication when virus is inoculated at multiplicities in excess of 10. This fusion occurs 'from without' and can be recognized within 2 to 6 h by inoculation of cells with either live or inactivated virus. Furthermore, cells which are incapable of supporting replication of the virus can be fused (Harter & Choppin, 1967). This syncytial type of cytopathic effect is also characteristic of the goat lentivirus infection of cell cultures (Narayan et al., 1980).

One of the enigmas of these virus infections of their animal hosts is that the cytopathic effects described above are never seen in infected tissues in vivo. Specific virus target cells are unknown, and neither exponential replication nor syncytia are seen in diseased target organs (brain, lung and joints) (Georgsson et al., 1977; Cork & Narayan, 1980). Pathological changes in these organs are associated with the cellular immune responses of the host (Nathanson et al., 1976). After inoculation of the animal, virus replication commences and is maintained at a minimal level in target tissues for months to years. Cell-free homogenates of these tissues have very little infectivity for fibroblastic cell cultures. However, when the tissues are explanted into culture, cellular outgrowths commence producing virus with concomitant development of cytopathic effect in a similar manner to that seen when sheep choroid plexus (SCP) cultures are inoculated with virus (Narayan et al., 1977).

In order to better understand the mechanisms of slow replication, we have used both the tissue culture-adapted Icelandic visna virus K 1514 and local field isolate viruses to study their replication in different target cells, specifically fibroblasts and macrophages, and to relate these findings to target cells in the infected animal.

**METHODS**

*Viruses.* Virus K 1514 which has been used routinely in this laboratory (Narayan et al., 1978) was used in this study as a reference Icelandic virus and cultivated in SCP fibroblasts. Field isolates were obtained from several naturally infected sheep with clinical signs of dyspnea, ataxia and arthritis, and from a congenitally infected goat. The viruses were obtained from these animals by harvesting supernatant fluids either from cultures of blood macrophages (see below) or from tissue explants. BL19 was obtained from macrophage cultures of a 5-year-old Border Leicester sheep with pneumonia. Progressive pneumonia viruses (PPV) 5 and 9 were derived from tissue explants of two sheep in Washington State. These animals had ataxia, pneumonia and a previously unrecognized arthritic disease (Oliver et al., 1981), and were kindly provided by Dr J. Gorham, U.S.D.A. Pullman, Wash., U.S.A. COR virus 101 was obtained from lung explants of a 3·5-year-old Corriedale sheep with both central nervous system and lung disease (Sheffield et al., 1980). COR virus 104 was obtained from another Corriedale ewe of the same flock. This animal had only interstitial pneumonia. SHROP virus was derived from blood macrophages of a 4-year-old Shropshire ewe with
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severe interstitial pneumonia. The goat agent was obtained from cultured blood macrophages of a congenitally infected Toggenburg kid.

**Cell cultures**

*Fibroblastic cultures.* SCP cell cultures, a fibroblastic cell line, were used to propagate K1514 as described previously (Narayan *et al.*, 1978). Goat synovial membrane cultures (GSM) are used routinely to propagate the goat field virus. These latter cells have macrophage-like properties in early subculture being phagocytic (Narayan *et al.*, 1980) and susceptible to carageenan toxicity (O. Narayan *et al.*, unpublished results). Multiple subcultivation of these cells give rise to fibroblastic cells which, similar to SCP, are not phagocytic and resist treatment with carageenan. Both GSM and the fibroblast cells were used in this study.

*Macrophage cultures.* Macrophage cultures were derived from peripheral blood leukocytes (PBL), lungs, spleen and/or lymph nodes of sheep and goats. PBL macrophage cultures were obtained from heparinized blood from which mononuclear cells were purified by centrifugation on Ficoll-Hypaque gradients. Alveolar macrophages were procured at autopsy by trans-tracheal pulmonary lavage using heparinized Ca$^{2+}$, Mg$^{2+}$-deficient Hanks’ salt solution. Macrophage suspensions from spleen and lymph nodes were prepared from teased cell suspensions which were washed several times in Hanks’ solution. Macrophage suspensions from PBL cells were suspended in RPMI 1640 medium + 20% autologous plasma or heat-inactivated lamb serum and 60 mm$^2$ tissue culture dishes were seeded with either 1 $\times$ 10$^6$ alveolar cells or 1 $\times$ 10$^7$ cells of the other cell suspensions. Non-adherent cells were removed from the cultures after 3 h incubation at 37 °C using three consecutive washes with Hanks’ solution. Medium was changed to RPMI 1640 + 1% lamb serum after macrophage monolayers had formed, usually about 1 day for alveolar cells and 7 to 10 days for peripheral blood cells. These adherent cells were shown to be macrophages by their morphology, resistance to trypsinization and ability to phagocytose sheep red blood cells (SRBC) sensitized with rabbit anti-SRBC serum.

*Virus cultivation.* Cultures of macrophages or fibroblasts in dishes were inoculated with different dilutions of virus and incubated at 37 °C for 2 h. Monolayers were then washed and maintenance medium replaced. Supernatant fluids from cultures were harvested at different periods after inoculation and assayed for infectivity. Infectivity titres of the sheep field virus suspensions were determined by inoculating normal macrophage cultures with 10-fold dilutions of virus. After 10 days, sheep fibroblast cells were added to the cultures and examined for fusion 7 to 10 days later. Assay of the goat virus in goat macrophage cultures was performed similarly by addition of goat fibroblasts to inoculated macrophage cultures as described above. Alternatively, supernatant fluids from these cultures were transferred to goat synovial membrane cultures in which the virus replicates productively with attendant cytopathic effect.

*Electron microscopy.* Alveolar macrophage cultures prepared from sheep infected with K1514 and PPV5, as well as sheep macrophages inoculated *in vitro* with BL19 virus, were selected for studies of virus morphogenesis. Fibroblast cells were also added to replicates of these cultures. All cultures were fixed *in situ* with a solution of 1% paraformaldehyde and 1.25% glutaraldehyde, and processed for electron microscopy by previously detailed techniques (Gilden *et al.*, 1978).

*Animal inoculations.* Two 6-month-old Corriedale sheep were inoculated intracerebrally with K1514, and two each with field viruses COR 101 and PPV5. Four young goats were similarly inoculated with the goat field isolate. All inoculations were performed using surgical procedures described previously (Narayan *et al.*, 1978). Each animal was inoculated in both cerebral hemispheres with 0.1 ml containing between 10$^6$ and 10$^8$ TCID$_{50}$ of the various
agents. These animals were killed approx. 6 weeks post-inoculation and tissues examined for virus content.

RESULTS

Comparison of replication of viruses in fibroblasts and macrophages

The basic strategy of this experiment was to compare the efficiency of replication of the field viruses with K1514 in SCP fibroblasts and sheep macrophage cell cultures. Agents grown in macrophage cultures were titrated in fibroblasts and macrophage cultures, and these were observed for development of cytopathic effect during the following 2 weeks. Fibroblasts were then added to the macrophage cultures, which were observed for a further 7 to 10 days. After one or more passages in fibroblastic cells, the viruses were also assayed for infectivity in fibroblasts and sometimes in macrophage cultures. These results are shown in Table 1 and the titres represent a range of at least three replicates for each assay.

It is evident that K1514 replicated to higher titres in fibroblast cultures than in macrophages. However, both cell types seemed equally sensitive to the infection. K1514 also differed in its cytopathic effects in the two cell types. Inoculation of SCP fibroblasts at multiplicities in excess of 10 caused ‘fusion from without’ and degeneration within 24 h without virus production. Inoculation at an m.o.i. of 5 resulted in maximum virus production and cytopathic effect (fusion with stellate cell formation) by day 3. Inoculation at an m.o.i. of $10^{-4}$ resulted in a delay of both cytopathic effect and virus yield. However, these cultures eventually produced as much infectious virus by day 10 as the preceding cultures inoculated at higher multiplicities. At peak production the K1514 virus yield was approx. $10^7$ TCID$_{50}$/ml of supernatant fluid. These virus titres fell precipitously as cell death from cytopathic effect progressed.

In contrast to fibroblasts, macrophages did not ‘fuse from without’ regardless of the m.o.i. of K1514. At high m.o.i. (100), the cells frequently died by day 10 post-inoculation although supernatant fluids never had more than $10^4$ fibroblast TCID$_{50}$. At lower m.o.i.s, these cultures did not develop cytopathic effects but continued to produce a constant amount of infectivity of $10^3$ to $10^4$ fibroblast TCID$_{50}$/ml for up to 3 weeks post-inoculation. Addition of fibroblasts to these inoculated macrophages resulted in massive cytopathic effect within a few days and amplification of virus titres to levels seen after direct inoculation of the fibroblast cells.

Replication of the field viruses

Similarly to K1514, none of these viruses caused cytopathic effect in macrophage cultures after inoculation at multiplicities of 1 or less. However, they all replicated to titres of $10^5$ to $10^6$ macrophage TCID$_{50}$/ml in these cultures and these levels of infectivity were maintained in supernatant fluids for up to 3 weeks post-inoculation. The infectivity titres were ascertained from the fusion of fibroblasts added to dishes of macrophages which had previously been inoculated with 10-fold dilutions of supernatant fluid.

Although fibroblasts underwent fusion after addition to infected macrophages, the direct inoculation of fibroblast cultures with the field viruses did not always cause cytopathic effects (Fig. 1). Titration of these agents in fibroblast cultures showed that the viruses either failed to replicate in the fibroblasts or did so only when inoculated at high multiplicities (1 to 10) (Table 1). In many cases, passage of infectious supernatant fluids through the fibroblast cultures resulted in further reduction of virus yield or even loss of infectivity in supernatant fluids, as determined by assay in the macrophage–fibroblast system. Electron microscopic examination of these fibroblasts did not show any evidence of virus infection and tests for
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Fig. 1. Morphological responses of fibroblasts and macrophages to infection with field viruses. (a) Fibroblasts inoculated with BL19 virus with no cytopathic effect. (b) Macrophages inoculated with BL19 virus with no cytopathic effect. (c) Co-culture of normal macrophages and fibroblasts with no cytopathic effect. (d) Co-culture of normal macrophages with infected fibroblasts shown in (a) with resultant fusion of the latter. Phase-contrast microscopy; all bar markers represent 50 μm.

Table 1. Comparison of the replication of Icelandic visna and field isolate viruses in fibroblastic (F) and macrophage (Mφ) cultures respectively, after cultivation in each of the two cell types*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Host cell culture</th>
<th>Fibroblasts</th>
<th>Mφ</th>
</tr>
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<tbody>
<tr>
<td>K1514</td>
<td>F</td>
<td>10^6–10^7</td>
<td>10^6</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10^2–10^3</td>
<td>10^4</td>
</tr>
<tr>
<td>BL19</td>
<td>F</td>
<td>10^5–10^6</td>
<td>10^3–10^4</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10^3</td>
<td>10^4</td>
</tr>
<tr>
<td>PPV5</td>
<td>F</td>
<td>10–10^2</td>
<td>10^3</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10–10^2</td>
<td>10^4</td>
</tr>
<tr>
<td>PPV9</td>
<td>F</td>
<td>10^2–10^3</td>
<td>10^5</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10^2</td>
<td>10^4</td>
</tr>
<tr>
<td>COR 101</td>
<td>F</td>
<td>N–10^2</td>
<td>10^4</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10^2</td>
<td>10^4</td>
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<tr>
<td>COR 104</td>
<td>F</td>
<td>N–10^2</td>
<td>N–10^2</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10^2</td>
<td>10^4</td>
</tr>
<tr>
<td>SHROP V</td>
<td>F</td>
<td>10^2–10^4</td>
<td>10^6</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10^2–10^4</td>
<td>10^6</td>
</tr>
<tr>
<td>Goat (GLV)</td>
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<td>N–10^2</td>
<td>N–10^2</td>
</tr>
<tr>
<td></td>
<td>Mφ</td>
<td>10–10^4</td>
<td>10^4</td>
</tr>
</tbody>
</table>

* Tenfold dilutions of each suspension were inoculated on replicates of each culture. Inoculated fibroblasts were examined directly for cytopathic effect and macrophages assessed by development of fusion of fibroblasts which were added after virus inoculation.

† Titres are reported as 50% endpoint dilutions causing cytopathic effects.

‡ N, Negative result.
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Table 2. Macrophage (Mφ) dependence of field virus expression in fibroblasts (F) *

<table>
<thead>
<tr>
<th>Parental Mφ + F-infected co-culture</th>
<th>F subculture†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion 4+</td>
<td>P1 P2 P3 P4 P4 + Mφ</td>
</tr>
<tr>
<td>Virus titre 10^5</td>
<td>4+ 2+ N N 4+</td>
</tr>
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</table>

* Fibroblasts added to BL19-infected macrophages underwent extensive fusion (4+: syncytia in every microscopic field).
† The fibroblasts were subcultured four times successively and each subculture examined for virus content in the supernatant fluid and cytopathic effect in the cells. Note lack of both from P2 onward. Virus activation with attendant cytopathic effect developed after P4 cells were added to normal macrophages.
‡ N, Negative result.

reverse transcriptase activity in supernatant fluids (as performed in a previous study, Narayan et al., 1980) were negative, even after 100-fold concentration of the fluids by ultracentrifugation (data not shown). The evidence therefore points to a minimal or a non-productive infection in the fibroblasts. However, addition of these inoculated non-producer fibroblasts to normal macrophage cultures resulted in prompt fusion of, and virus production by, the fibroblasts. Furthermore, progeny cells of the non-producer fibroblasts, after four consecutive subcultivations at dilutions of 1:4, still underwent fusion with production of 10^4 TCID_{50}/ml after co-cultivation with normal macrophages. Representative cultures are shown in Fig. 2. Macrophages, therefore, seemed indispensable for the expression of virus and induction of cytopathic effect in fibroblasts, although it is evident that non-productive infection could readily occur by direct inoculation of fibroblasts with virus.

Whether virus-producing fibroblasts required the continuous presence of macrophages for virus expression was determined in two experiments. First, a series of macrophage cultures was inoculated with BL19 virus and incubated for 5 days. Supernatant fluids from these cultures had approx. 10^5 macrophage TCID_{50}/ml but failed to cause cytopathic effect in fibroblasts. One of the macrophage cultures was irradiated with U.V. light and fibroblasts were then added to this as well as the unirradiated dishes. Syncytial formation of fibroblasts commenced in all the co-cultures within 4 h after addition to the macrophages. These early syncytia with about six to eight nuclei gradually enlarged during the following 10 days to giant cells containing 50 to 100 nuclei. Fibroblasts added to the irradiated macrophages developed the early fusion but this did not progress to the large syncytia which developed in unirradiated infected macrophages. Co-cultivation of normal fibroblasts with uninfected macrophages did not result in fusion.

The second experiment took advantage of the resistance of macrophages to trypsinization. Fibroblasts with extensive syncytial formation in an infected macrophage–fibroblast co-culture were subcultured to determine whether they would continue producing virus in the absence of macrophages. These fibroblasts (passage 1, P1) were seeded in two flasks, one of which was maintained for 6 weeks. The other was subcultured again into two flasks (P2) at approx. 20-day intervals when the cells had reached confluence. At P4 the cells were then added back to a normal macrophage culture as shown in Table 2. Nearly all the cells in the P1 culture were involved in syncytia at the time they were plated. However, at 6 weeks most

Fig. 2. Ultrastructural appearance of a sheep macrophage which had been inoculated in culture 7 days previously with BL19 virus. Approx. 10% of all cells viewed by electron microscopy contained virus particles. Most particles were located within smooth membrane vacuoles which were either multipie (a) or single (c) for individual macrophages. The intricate arrangement of the vacuoles from the macrophage shown in (a) is seen in detail in (b). Bar markers represent 5 μm, 1 μm and 0.5 μm in (a), (b) and (c) respectively.
of the syncytia had been replaced by cells with single nuclei and infectivity of the supernatant fluid had decreased from $10^5$ to $10^2$ macrophage TCID$_{50}$/ml. Approx. 50% of the P2 cells were involved in syncytia at 20 days but no infectivity was found in the supernatant fluid. Cultures at P3 and P4 lacked syncytia and had no infectivity in their supernatant fluids. Replacement of the supernatant fluid from a P4 culture with that from an uninfected macrophage culture failed to induce virus production during a 10 day observation period. However, addition of P4 cells to normal macrophages resulted in typical fusion and virus production by the fibroblasts. These experiments showed that fibroblasts could be infected with virus but virus expression was dependent on the presence of macrophages. The latter cells were not only indispensable for the initiation of expression of virus by fibroblasts, but also had to be present constantly for continuation of virus production.

There was no obvious functional defect in any of the infected macrophage cells since they all readily phagocytosed antibody-coated red blood cells.

**Virus recovery from infected animals**

In all cases virus cytopathic effect was identified when PBL from infected sheep and goats were co-cultivated with the fibroblast cells. Cell-free tissue homogenates of spleen, lung, lymph node and brain did not cause cytopathic effect in fibroblasts. However, explants of all these tissues developed cytopathic effects. These results thus confirmed previous findings of minimal amounts of free virus in infected tissues and the ‘activation’ of virus by explantation (Petursson et al., 1976; Narayan et al., 1977; Cork & Narayan, 1980).

Non-adherent cells from PBL did not have infectivity for macrophages or fibroblasts from either of the two species. None of the macrophage cultures derived from PBL, spleen, lung and lymph node of the infected animals developed cytopathic effect. However, these cells produced virus continuously during a 3 week observation period. The levels of infectivity in the supernatant fluids of the cultures closely resembled those of macrophage cultures inoculated in vitro (Table 1).

**Ultrastructural observations**

Three types of cultures were examined by electron microscopy: (i) cultured alveolar macrophages from a sheep infected with PPV5; (ii) PBL-derived macrophages obtained from
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a normal sheep and infected in culture with BL19 virus; (iii) a BL19-infected macrophage–
fibroblast co-culture in which the fibroblasts had undergone fusion. Examination of the
macrophages from all three types showed that only a small proportion of the cells contained
virus particles. These cells had no evidence of cytopathic changes although virions in variably
sized vacuoles were often found widely distributed in the cytoplasm (Fig. 2a to c). Occasionally, virions were recognized in the process of budding from the membrane of the
vacuoles. Notably, no virus maturation was seen at the cell membrane of these macrophages.
In the macrophage–fibroblast co-culture, the intracellular location of virions in the
macrophages was the same as that seen in pure cultures of infected macrophages. However,
numerous morphologically intact virions were seen at and budding from the surface of the
fibroblasts (Fig. 3).

DISCUSSION

This study shows that the ovine–caprine lentiviruses, which cause slow diseases in different
organ systems of their natural hosts, preferentially infect and replicate in macrophages,
causing non-cytopathic, persistent infections of these cells. The combination of this low-grade
productive infection of macrophages with a non-productive infection of a non-macrophage
cell type provides a good parallel for restricted virus replication in the animal and may be the
key to understanding the phenomenon of this 'slow infection'. The results agree with, and
partially explain our previous finding that all virus genomes are not expressed in tissue cells of
inoculated sheep (Haase et al., 1977). This concept has been reconfirmed and extended in
subsequent studies by Brahic & Haase (1978) and Brahic et al. (1981) in which restriction of
the virus life-cycle in certain brain cells of intracerebrally inoculated sheep occurs at the level
of transcription of the virus RNA.

Although prototype Icelandic visna virus K1514 causes rapid cytolysis of fibroblasts, its
replication in the animal was mirrored by its replication in macrophage cultures in which
relatively small quantities of virus were produced for a long period without cytopathology.
This suggests that the virulence of this virus for fibroblasts may be the result of
cultivation-selection in vitro for a cytopathic virus and may have little relevance to the events
in the infected animal. The use of field isolate viruses from natural cases of infection probably
provides a more accurate reflection of the mechanisms underlying this persistent infection.
The preferential replication of the field viruses in macrophages and the non-cytopathic nature
of this infection may provide an ideal mechanism for virus persistence in the animal since
clearance by the reticuloendothelial system is effectively subverted. In this respect these
ovine–caprine lentiviruses share a common pathway for persistent infection with a number of
other viruses which also infect macrophages. These viruses include other retroviruses such as
the agent of equine infectious anaemia (Crawford et al., 1978), avian leukosis (Gazzolo et al.,
1979) and Friend virus-induced erythroleukaemia (Marcelletti & Furmanski, 1979); certain
of the flaviviruses, including West Nile virus (Peiris & Porterfield, 1979), and dengue viruses
(Halstead, 1979); Aleutian disease virus of mink (Porter et al., 1980), lactic dehydrogenase
virus (Rowson & Mahy, 1975) and African swine fever virus (Coggins, 1974). One of the
main differences between most of these agents and the lentiviruses reported here is the
restricted level of lentivirus synthesis by infected macrophages. A further difference of course
is the fact that the non-macrophage cells may be non-productively infected as seen in
cultivated fibroblasts. Such cells in vivo with unexpressed virus genomes would also be spared
from immune elimination as suggested by Brahic et al. (1981).

In addition to possible differential restriction of virus replication by cells in vivo and in
vitro, the unique virus–macrophage–fibroblast interaction provides a better understanding of
virus activation when infected tissues are cultivated in vitro. Thus, the minimal quantities of
infectivity usually observed in tissue suspensions from infected animals could be explained
either by a negligible amount of infectivity in cell-free suspensions or the lack of infectivity of field viruses for the fibroblast cultures used as indicators. Support for the latter hypothesis comes from examination of cell-free lung homogenates from the Shropshire and the two Corriedale sheep with severe interstitial pneumonia. These homogenates had approx. $10^5$ macrophage TCID$_{50}$ but less than $10$ fibroblast TCID$_{50}$ (O. Narayan et al., unpublished results). The greater sensitivity of the explantation method may be a reflection of two interdependent factors. First, macrophages are always present in outgrowths of tissue explants and secondly, explant outgrowths invariably give rise to fibroblasts. Explant cultures, therefore, would automatically provide the conditions for obtaining fusion, since virus-infected macrophages would cause fusion of fibroblasts.

The non-productive infection of sheep fibroblasts with the goat virus (GLV) and the rescue of this agent by phagocytic synovial membrane cells (GSM) had been observed in a previous study (Narayan et al., 1980). However, these data were not evaluated from the perspective that macrophages were virus target cells. In the present study, the mechanism of macrophage induction of virus synthesis in infected non-producer fibroblasts and the reason for the obligatory presence of the macrophages for the continuation of this process still remain to be defined. Results so far show that this activation factor was not present in the supernatant fluid of macrophages. However, the fact that irradiated infected macrophages had the capacity to fuse fibroblasts shows that the factor is cell-associated. Possibly, it is an enzyme which may have an obligatory function in cleavage of virus precursor protein(s) in infected fibroblasts into smaller units required for virion assembly. This analogy is drawn from studies of paramyxoviruses in which maturational defects can be corrected by purified proteases (Scheid & Choppin, 1974; Nagai et al., 1976) and by macrophages (Waters et al., 1974). Whatever the mechanism, the inference drawn for pathogenesis is that the virus-infected macrophage could determine the host-cell range of the virus and thus determine target organs for disease.

The site of replication of these viruses in macrophages and fibroblasts provides grounds for further speculation on the pathogenesis of the diseases caused by these agents. Since virus particles are formed predominantly within cytoplasmic vacuoles of macrophages, these cells may elude detection by cellular surveillance systems and therefore escape immune elimination. However, macrophage-dependent virus morphogenesis in fibroblasts which express virus antigens on the cell surface would be ideal targets for immune response. If parenchymal cells of target organs replicate virus similar to fibroblasts in culture, then the conditions would be fulfilled for the immunopathological lesion. A chance fusing reaction between a brain cell, a pneumocyte, or a synovial cell and an infected macrophage might result in enough expression of virus proteins for the cell to be recognized by the host immune system. Invasion of the neurophil, lung and joints with inflammatory cells, including more infected monocytes, would then follow as part of an immune-mediated pathology (Panitch et al., 1976). This could result in amplification of the local infection and lead to the encephalitic and pneumonic and arthritic lesions which characterize natural visna/maedi/arthritis.

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