Precipitating antibodies in experimental visna and natural progressive pneumonia of sheep


Serological responses of Icelandic sheep experimentally infected with visna virus (VV) were contrasted with responses in American Targhee sheep naturally infected with progressive pneumonia virus (PPV). Precipitating antibodies assayed by immunodiffusion were compared with the neutralising and complement fixing antibody response. In experimental infections with VV, complement fixing and neutralising antibodies appeared early after infection and rose to high levels in all sheep, while precipitating antibodies were detected only at minimal titre. In natural infections with PPV, immune responses were less consistent and precipitating antibodies were detected more frequently than complement fixing or neutralising antibodies against PPV. These results may suggest important biological differences between the lytic fibroblast-tropic virus strains used for experimental infection of Icelandic sheep and the nonlytic macrophage-tropic strains of PPV circulating in nature. Lytic strains evoke a brisk response against the viral glycoprotein with high titre neutralising antibody while nonlytic strains induce a less consistent response to the glycoprotein.

VISNA virus (VV) and progressive pneumonia virus (PPV) are synonyms for an enzootic retrovirus of sheep which causes a slow infection and chronic disease of the lungs (progressive pneumonia, maedi or zwoegerziekte) and central nervous system (visna) (Kimberlin 1976, Petursson et al 1976, Georgsson et al 1980, Brahic and Haase 1981, Nathanson et al 1983, Narayan et al 1983).


The authors undertook a collaborative study to determine whether the differences in serological responses in experimental and natural infections, were due to variations in the methods or to differences in the infectious process. The results indicate that there are important biological differences between laboratory strains and field isolates of VV and PPV which influence both the character of the immune response and the outcome of certain laboratory tests.

Materials and methods

Virus and antigens

VV, strain 1514, and PPV were grown in sheep choroid plexus (SCP) cells, as previously described (Petursson et al 1976, Narayan et al 1983).

Supernatant fluids harvested from infected SCP cultures were used for neutralisation tests and as antigen in complement fixation tests. For routine immunodiffusion tests supernates were concentrated 100-fold by dialysis. For the immunodiffusion tests presented in Table 1, antigens were also prepared from virus banded on sucrose gradients or from infected SCP cells scraped from flasks and sonicated (Terpstra and de Boer 1973).

Sera

Several sources of antisera were used. (a) A panel of 70 samples, representing three age groups, was obtained from a flock of Targhee sheep, maintained in Idaho, and known to have a high level of endemic PPV infection (Gates et al 1978). (b) A group of 19

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Icelandic sheep, known to be free of natural infection, were injected by the intracerebral route with 10^6 TCID50 of vv, strain 1514, and bled periodically (Petursson et al 1976). (c) A hyperimmune serum was obtained from a Hampshire sheep (703) which had received numerous intramuscular injections of vv, strain 1514, in complete Freund’s adjuvant. (d) An antisera was obtained from a goat hyperimmunised with purified p25 protein of vv, strain 1514. (e) An antisera against the glycoprotein was obtained from a guinea pig hyperimmunised with glycoprotein of vv. Both anti-p25 and anti-glycoprotein sera immunoprecipitated single proteins when reacted with disrupted virions (Pyper et al 1984).

**Immunodiffusion tests**

An agarose gel diffusion system was used, consisting of 0.5% agarose in 8% saline. Wells were cut in a hexagonal pattern and virus or serum was added in a volume of 0.05 ml per well. Just before use, virus was treated with 0.2% per cent sodium dodecyl sulphate detergent for 15 minutes. Immunodiffusion plates were incubated at room temperature in a humidified atmosphere and scored at 24 to 48 hours.

**Neutralisation tests**

For tests with PPV, a micro-neutralisation system was used. Serial twofold dilutions (0-2 ml) of sera were prepared in tubes. To these were added 0-2 ml (100 TCID50 1.0-05 ml^-1) of virus, and the mixtures were incubated overnight at 4°C. The following day 0-1 ml of each dilution was plated in two replicates onto 96-well microtitre plates containing a monolayer of sheep choroid plexus cells. Plates were observed daily for up to two weeks for evidence of cytopathic effect. For tests with vv, a tube neutralisation test was used as described previously (Petursson et al 1976).

**Complement fixation tests**

Complement fixation tests were conducted according to a published protocol (Petursson et al 1976). Viral antigen (0.025 ml) was reacted with an equal volume of serially diluted test serum in 96-well round bottom microtitre plates. To each mixture was added 0.05 ml of guinea pig complement (four 50 per cent haemolytic units) followed by incubation at 4°C overnight. Following incubation, 0.05 ml of a suspension of sensitised sheep erythrocytes was added and the plates were placed at 37°C for 30 minutes; they were then centrifuged and haemolysis was scored visually.

**Results**

**Precipitating antibodies assayed by immunodiffusion**

There are two precipitin lines which can be distinguished in the immunodiffusion test, representing the glycoprotein and the major internal antigen (p25). These two precipitin lines are shown in Figs 1 and 2 together with evidence for their identity. Concentrated supernatant from visna-infected cell cultures, when treated with detergent (NP-40 or sodium dodecyl sulphate), yields both antigens and was used for the tests shown in Figs 1 and 2. When a hyperimmune anti-vv serum is used both lines are seen. The line furthest from the antigen well is the p25 and its identity is confirmed by an anti-p25 monospecific antisera (Fig 1). The line closest to the antigen well is the glycoprotein line; its identity is confirmed by a monospecific antisera against glycoprotein which, when added to the antigen well, complexes with the glycoprotein and deletes the precipitin line closest to the antigen well (Fig 2).

Table 1 shows that the relative concentration of the two antigens differs markedly depending upon the method used to prepare antigen. The table also indicates some differences in the properties of p25 and glycoprotein. p25 may be obtained from purified virions, concentrated infected cell culture supernatant or disrupted infected cell pack; concentrated supernatant is the best source of glycoprotein. Glycoprotein is less stable than p25 and is rendered non-antigenic by treatment with 0-2 per cent sodium dodecyl sulphate and ether (30 minutes at room temperature). Antigens prepared from vv and PPV give lines of identity in the immunodiffusion system (data not shown).

For diagnostic purposes each immunodiffusion plate included a reference antisera producing two precipitin lines. With this reference reagent it was easy...
to identify which protein was precipitated by antisera with a single specificity (Fig 1).

**Sero logical responses in Targhee sheep with natural PPV infection**

A panel of 70 sera was assembled to represent three age groups from a flock of Targhee sheep from Idaho known to be heavily infected with PPV. Fifty-six of 70 sera were classified as ‘infected’ because they were positive in one or more tests (Table 2).

From Table 2 it can be seen that no single test detects more than two-thirds of infected sheep. Furthermore, the immunodiffusion test for glycoprotein is more sensitive than the complement fixation test or neutralisation test (using PPV), both of which detect less than 30 per cent of infected animals. Results with the neutralisation test depend upon the virus used; this test is more sensitive with the highly cytopathic 1514 strain of vv, in part because a much smaller virus input produces definite cytopathic effect in SCP cells.

**Serological responses in Icelandic sheep with experimental vv infection**

A group of Icelandic sheep were infected with the 1514 virus, a laboratory-selected cytopathic strain of vv. Table 3 shows comparative serological responses in these animals. Complement fixing antibody appeared at one to two months, followed shortly thereafter (three to four months) by neutralising antibody. All sheep developed high titres of both antibodies. Precipitating antibody to glycoprotein appeared simultaneously with neutralising antibody but titres were minimal since most sera, when diluted twofold, failed to precipitate antigen. Precipitating antibody to p25 could only be detected in about 40 per cent of infected animals; again, titres were minimal.

**Discussion**

It is clear that the immunodiffusion test can detect, in the sera of infected sheep, precipitating antibodies against two of the proteins of vv or PPV; the glycoprotein which is the major envelope protein and the
TABLE 2: Relative sensitivities of different serological tests in American Targhee sheep naturally exposed to progressive pneumonia virus

<table>
<thead>
<tr>
<th>Test</th>
<th>Percentage positive</th>
<th>Infected</th>
<th>Uninfected</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(58 sera)</td>
<td>(14 sera)</td>
</tr>
<tr>
<td>ID (gp)</td>
<td>64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ID (p25)</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CF (VV)</td>
<td>27</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VT (PPV)</td>
<td>28</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VT (VV)</td>
<td>69</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Approximately equal numbers of sera were obtained from sheep aged one to two, three to five and six to eight years, selected so that about half in each group had precipitating anti-glycoprotein antibody. Infected: positive in one or more tests

ID Immunodiffusion for precipitating antigen
gp Glycoprotein
p25 Major internal antigen
CF Complement fixation
NT Neutralisation test
VV Viscus virus
PPV Progressive pneumonia virus

p25 which is the major core protein. Similar conclusions regarding the identity of the two precipitating antigens of maedi-visna virus have been published by others (Cutlip et al 1977a, Larsen et al 1982a). Parallel findings have been reported for the bovine leukaeima system (Onuma et al 1975, Miller and Van der Maaten 1976, Rohde et al 1978).

If reference sera producing both precipitin lines are included in the test (Fig 1), it is readily possible to specify the precipitating antibody in each positive serum. There are a number of reports (Terpstra and de Boer 1973, Cutlip et al 1977a, 1977b, 1979, Gates et al 1978, Larsen et al 1982a, 1982b) of precipitating antibody responses in sheep infected with VV or PPV. However, some of these studies (Terpstra and de Boer 1973, Cutlip et al 1977b) are difficult to interpret because it was not stated whether one or both precipitating antibodies were being detected. In part this may have been due to the method of antigen preparation (untreated tissue culture supernatant or infected cell pack) used in some of these studies which probably failed to yield adequate amounts of glycoprotein or p25 (Table 1).

The results of this collaborative study clearly indicate that there are major differences between the serological responses in experimental visna and natural progressive pneumonia. Icelandic sheep raise consistent neutralising and complement fixing antibodies, both of which appear within two to four months after infection. In natural infections, the frequency of complement fixing and neutralising responses is less than 30 per cent, even though most animals have presumably been infected for many months (Gates et al 1978). Conversely, the immunodiffusion test for glycoprotein detects a higher proportion of natural infections (about 65 per cent), than do neutralisation and complement fixation assays. This explains the preference of field workers for the immunodiffusion test (Cutlip et al 1977a, 1977b, Gates et al 1978), even though it appears relatively insensitive when applied to experimental visna.

It seems likely that an important biological difference underlies these divergent serological responses. Narayan et al (1982) have documented major differences in strains of VV and PPV as to their virus-cell interactions. Virus strains (such as 1514) used in Iceland were selected for their ability to produce severe cytolysis in fibroblasts (scp cells) with concomitant high titres of cell-free virus. Conversely, fresh field isolates of PPV show quite different properties, since they grow preferentially in macrophages rather than fibroblasts, induce fusion rather than lysis and yield much lower titres of cell-free virus when grown in fibroblasts.

Narayan et al (1983) have found that when field isolates of PPV were used to initiate experimental infections, the animals raise little or no neutralising antibody in marked contrast to the brisk neutralising antibody response evoked by the lytic strain 1514 of VV in either Icelandic or American sheep (Petursson et al 1976, Narayan et al 1978, 1984, Griffin et al 1978). It may be speculated that in vivo the lytic virus is released in higher titre and induces a more active immune response to the viral glycoprotein. In addition to biological differences between laboratory passed VV and field isolates of PPV, differences in breed of sheep (Targhee versus Icelandic) and in route and dose of infecting inoculum may have also contributed to the results reported here. In any event,

TABLE 3: Serum antibody responses of 19 Icelandic sheep experimentally infected with visna virus

<table>
<thead>
<tr>
<th>Months after infection</th>
<th>Percentage positive by test</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ID (gp)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>21</td>
<td>95</td>
</tr>
<tr>
<td>24</td>
<td>95</td>
</tr>
<tr>
<td>27</td>
<td>89</td>
</tr>
</tbody>
</table>

* A dose of 10⁶ TCID₅₀ was administered intracerebrally
ID Immunodiffusion test for precipitating antigen
gp Glycoprotein
p25 Major internal antigen
CF Complement fixation
NT Neutralisation test
Percentage positive of 19 sera tested
it appears that there is an important biological basis for the contrasting serological responses seen in experimental Icelandic visna and natural progressive pneumonia.

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References


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