Onderstepoort J. vet. Res., 55, 127–133 (1988)

THE POSSIBLE INVOLVEMENT OF IMMUNOSUPPRESSION CAUSED BY A LENTIVIRUS IN THE AETIOLOGY OF JAAGSIEKTE AND PASTEURELLOSIS IN SHEEP

M. S. MYER[1], H. F. A. K. HUCHZERMEYER[2], D. F. YORK[2], P. HUNTER[2], D. W. VERWOERD[2] and HELEN M. GARNETT[3]

ABSTRACT

MYER, M. S., HUCHZERMEYER, H. F. A. K., YORK, D. F., HUNTER, P., VERWOERD, D. W. and GARNETT, HELEN, M., 1988. The possible involvement of immunosuppression caused by a lentivirus in the aetiology of jaagsiekte and pasteurellosis in sheep. *Onderstepoort Journal of Veterinary Research*, 55, 127–133 (1988).

A South African isolate of ovine lentivirus was shown to cause a mild immunosuppression in sheep, reflected by a reduced delayed hypersensitivity reaction. This effect, measured in terms of skin swelling after intradermal inoculation with tuberculin, showed a positive linear relationship with the latency period before the appearance of jaagsiekte symptoms in animals co-infected with JSRV, as well as with the activity of monocytes. In a parallel study, increased susceptibility of lentivirus-infected sheep to infection with *Pasteurella haemolytica* was demonstrated. It is concluded that the lentivirus may play an enhancing role in both viral and bacterial infections of sheep by compromising the host's cellular immune response.

INTRODUCTION

In jaagsiekte (ovine pulmonary adenomatosis), transformation of the alveolar epithelial lining is caused by the jaagsiekte retrovirus (JSRV), a member of the *oncovirinae* subfamily (Verwoerd, Tustin & Payne, 1985). The tumour can be transmitted efficiently by intratracheal inoculation of new-born lambs with the virus (Verwoerd, Williamson & De Villiers, 1980; Sharp, Angus, Gray & Scott, 1983). However, considerable variation in the length of the latent period following infection, is characteristic of the transmission.

In many parts of the world jaagsiekte (JS) is closely associated with maedi, a chronic interstitial pneumonia caused by another retrovirus, maedi-visna virus (MVV), which belongs to the subfamily *lentivirinae* (Wandera, 1971; Palsson, 1976; Snyder, De Martini, Ameghino & Caletti, 1983; Markson, Spence & Dawson, 1983; Houwers & Terpstra, 1984). In South Africa a non-pathogenic lentivirus (SA-OMVV) was recently isolated and shown to be consistently present in jaagsiekte-infected lungs (Payne, York, De Villiers, Verwoerd, Quérat, Barben, Sauze & Vigne, 1986). This virus was found to be related to but not identical with maedi-visna virus (Quérat, Barban, Sauze, Vigne, Payne, York, De Villiers & Verwoerd, 1987).

The co-existence of the 2 retroviruses in a single animal has led to considerable speculation on the possibility of a synergism between them (Payne *et al.*, 1986; De Martini, Rosadio, Sharp, Russell & Lairmore, 1987). It has indeed been shown that whereas virus replication is restricted in experimental infections with visna virus (Geballe, Ventura, Stowring & Haase, 1985), it could be transmitted efficiently to a natural case of jaagsiekte, with active replication of the lentivirus in the lungs (Dawson, Venables & Jenkins, 1985). As visna virus is known to replicate *in vitro* in macrophages (Narayan, Kennedy-Stoskopf, Sheffer, Griffin & Clements, 1983; Payne *et al.*, 1986), one possible explanation for this observation is that the increase in the numbers of alveolar macrophages in jaagsiekte provides a larger reservoir of cells for the replication of lentivirus (De Martini *et al.*, 1987). This increase is believed to be caused by a chemotactic factor secreted by JS-tumour cells (Myer, Verwoerd & Garnett, 1987 a). The *in vitro* interaction of these macrophages with other lymphoid cells results in the production of a factor which stimulates the growth of JS tumour cells, thus creating the possibility of further macrophage accumulation (Myer 1987; Myer, Verwoerd & Garnett, 1987 b).

An alternative explanation would be an immunosuppressive effect by JSRV, similar to that of Mason-Pfizer monkey virus to which it seems to be related (Sharp & Herring, 1983).

On the other hand, some lentiviruses are known to exert a powerful immunosuppressive effect. The best example of this is the human immunodeficiency (HIV-I) or AIDS virus (Popovic, Sarngadharan, Read & Gallo, 1984). It was therefore decided to investigate whether lentivirus-mediated immunosuppression plays a role in the etiology of jaagsiekte.

MATERIALS AND METHODS

Viruses

JSRV was obtained and stored as lung rinse pellets from experimentally produced jaagsiekte cases as previously described (Verwoerd, Payne, York & Myer, 1983). These pellets were shown to contain both JSRV and lentivirus (SA-OMVV).

Lentivirus was isolated by culturing alveolar macrophages from experimental jaagsiekte cases for 1–2 weeks. Lung rinse fluid was centrifuged at low speed (400 × g for 30 min), the sediment resuspended in medium and the macrophages allowed to adsorb to the surface of plastic cell culture flasks for 120 min at 37 °C. Medium was replaced at 3-day intervals and tested weekly for the presence of reverse transcriptase (RDP) activity (Verwoerd *et al.*, 1983). Lentivirus positive medium was centrifuged (54 000 × g for 90 min) and the resulting pellet used directly, or for infection of sheep choroid plexus cells (Payne *et al.*, 1986).

Infection of lambs

Experimental lambs were inoculated intratracheally within one week of birth with either SA-OMVV alone, or with pellets containing both JSRV and SA-OMVV. Virus concentrations were estimated by means of the RDP assay and adjusted to contain 1×10^6 RDP units in a 4,0 mℓ dose. The two groups of animals were kept under semi-isolated conditions to prevent cross-contamination.

[1] P.O. Box 28203, Sunnyside, Pretoria, South Africa, 0132

[2] Veterinary Research Institute, Onderstepoort 0110, South Africa

[3] Department of Biology, University of Wollongong, Northfield Avenue, Wollongong, NSW, Australia, 2500

Received 23 March 1988—Editor

Skin sensitization assay

The *in vivo* cellular immune response of experimental animals was tested by means of a delayed hypersensitivity skin test. After sensitizing the sheep with 10 mg of killed *Mycobacterium avium*[1] in 5 mℓ of liquid paraffin injected intramuscularly, 0,1 mℓ of PPD of avian tuberculin[2] was inoculated intradermally 6 weeks later. The thickness of folded skin was measured at 0 h, 48 h and 72 h using a Hauptner caliper. In the case of JS-sheep, the sensitization was started as soon as clinical symptoms of the disease were seen, in order to measure the animals' cellular immune response at a comparable stage in the development of the tumour.

Isolation of monocytes

Monocytes were isolated from the peripheral blood of sheep essentially according to the method of Carlson & Kaneko (1973). Blood collected in 2 heparinized vacutainer tubes per sheep was centrifuged for 30 min at 1 000 × g. The buffy coats were pooled and resuspended in 4 mℓ of Dulbecco's phosphate buffered saline (PBS). Remaining red blood cells were lysed by the addition of 20 mℓ of distilled water, followed by the restoration of isotonicity by adding 10 mℓ of PBS containing 2,7 % NaCl after 30 s of gentle mixing. The white blood cells were centrifuged (400 × g for 10 min), washed once in PBS and then resuspended in F-12 nutrient medium[3]. The monocytes were subsequently removed from the white blood cell fraction by adherence to a plastic substrate for 2 h at 37 °C. Fresh F-12 nutrient medium was added to these tissue culture flasks and the adherent monocytes were dislodged by vigorous shaking.

Thymidine incorporation assay

Monocyte suspensions were added to the upper compartments of Modified Boyden Chambers. Glass fibre membranes separated upper and lower compartments of the chambers, where the latter compartment contained F-12 nutrient medium with foetal calf serum serving as growth stimulant. The monocytes, which adhered to the glass fibre membranes, were seeded at 8 × 10^5 cells/upper compartment/200 μℓ F-12 nutrient medium. ^{3}H-Thymidine (specific activity 21 Ci/mmol)[4], was added at the same time to each Boyden Chamber at 1 × 10^7 Ci/upper compartment/0,5 mℓ F-12 nutrient medium and the experiment allowed to run for 72 h in a 5 % CO_2 incubator at 37 °C. The assay was terminated by removing the membranes from the chambers and washing them by gentle swirling in PBS before placing them in glass scintillation vials, where the adherent monocytes were lysed with 250 μℓ 1 % sodium dodecyl sulphate per membrane. Each lysed sample was dissolved in Beckman Ready-Solve TM scintillation cocktail and read on a Beckman LS 9000 Scintillation counter.

In vitro *infection of alveolar macrophages with lentivirus*

Alveolar macrophages (AMs) isolated from the broncho-alveolar lavage performed on the excised lungs from a normal, healthy sheep, as described in Myer, Verwoerd & Garnett (1987a), were seeded into two sets of plastic tissue culture flasks. Each set consisted of 2 flasks containing 2 × 10^6 cells/flask/10 mℓ F-12 nutrient medium. One set of flasks was inoculated with 25 % RDP-positive supernatant from a previous AM culture producing lentivirus (4,5 × 10^7 cpm/flask) while the other set was cultured in F-12 nutrient medium only. After 24 h incubation at 37 °C, supernatants were discarded from both sets of flasks. The flasks were rinsed 3 times in PBS before addition of fresh F-12 nutrient medium. After incubation for 48 h, the supernatants were collected and assayed for RDP activity as described previously.

Chemotaxis assay

Assays for the chemotactic activity of various macrophage cultures were done as previously described (Myer *et al.*, 1987a). Briefly, the chemoattractant used was either conditioned medium from 15.4 tumour cell cultures or N-formyl-L-methionyl-L-phenyl-alanine (N-FMet)[5]. A stock solution of 10^{-5}M N-FMet was diluted 1:25 in F-12 nutrient medium prior to use in the assay and arbitrarily referred to as 100 % N-FMet. Varying concentrations of this solution was added to the lower compartments of modified Boyden chambers, which were separated by 10 μm pore size polycarbonate membranes[6] from the upper compartments containing 10^5 macrophages. After 3 h of incubation at 37 °C, the distance that the cells had migrated into each membrane was measured microscopically as described by Myer *et al.*, 1987a.

Infection with Pasteurella haemolytica

A *P. haemolytica* Type I strain, originally isolated from a field case, was passaged twice in mice to increase virulence before inoculation into serum broth for overnight culture. It was then transferred to 200 mℓ of tryptose phosphate broth[7] containing 0,5 % glucose. After incubating overnight at 37 °C on a rotary shaker, it was transferred to fresh tryptose-glucose broth in which it was grown for another 6 h. The bacteria were then harvested by centrifugation (800 × g for 60 min), washed and suspended in 10 mℓ of saline. Plate counts were performed to determine the concentration of viable organisms. Both lentivirus-infected and control animals were inoculated intratracheally with 1 × 10^{11} organisms in a volume of 10–20 mℓ, according to the size of the animal. Temperatures were taken daily for a period of 3 weeks and all animals observed for clinical signs of respiratory distress.

RESULTS

Cellular immune response in jaagsiekte-infected sheep

When jaagsiekte is transmitted to new-born lambs by intratracheal inoculation with pellets containing both JSRV and SA-OMVV, the latent period before clinical signs appear varies between 3 months and a year. In a few individuals no symptoms are seen after a year and only small, localized lesions are revealed by necropsy. In order to test whether suppression of the cell-mediated immunity (CMI) could be responsible for this variation in susceptibility, the delayed hypersensitivity response of each sheep was measured by means of a tuberculin skin test as described under MATERIALS AND METHODS. The 48 h differential measurements are shown in Fig. 1 as a function of the time of onset of jaagsiekte symptoms. The regression curve, fitted to the data according to Dunn (1977), clearly indicates a linear relationship, suggesting that there is a positive correlation between the latency period of clinical jaagsiekte and the CMI of the animal.

[1] Strain 20485 local strain:—isolated from porcine lymph node

[2] Produced at the Veterinary Research Institute, Onderstepoort, from *M. avium* strain D4, containing 25 000 tuberculin units/1 mℓ

[3] F-12 nutrient medium: F-12 medium (Ham, 1965) supplied by Highveld Biological (Pty) Ltd, Northway, Sandton, Johannesburg, South Africa, and containing 10 % foetal calf (supplied by the same company), penicillin, streptomycin and nystatin.

[4] Amersham International, Ltd., Amersham, England

[5] Sigma Chemical Company, St. Louis, USA

[6] Nucleopore Corporation, Pleasanton, CA 94566-3294 USA

[7] DIFCO

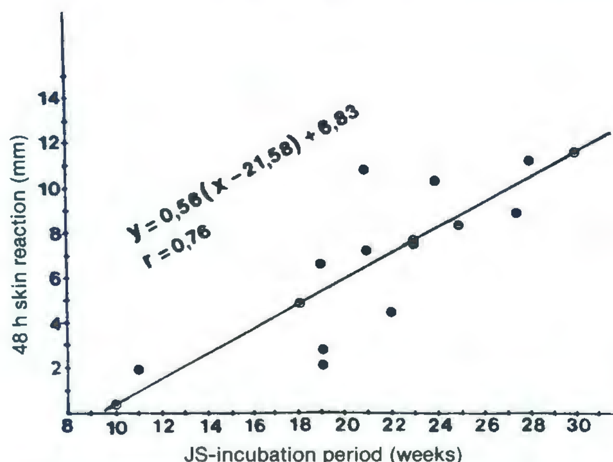

FIG. 1 Regression curve of skin sensitivity (mm) vs. jaagsiekte (JS) incubation period

Immunosuppressive activity of the 2 viruses

The immunosuppression accompanying the development of jaagsiekte lesions could be due to either or both of the retroviruses. In Fig. 2, the average skin reactions are compared in 3 groups of sheep: normal control animals, sheep infected with lentivirus only and sheep infected with both lentivirus and JSRV. A significant suppression of the delayed hypersensitivity reaction is seen after lentivirus infection, with possibly an additional effect when JSRV also is present.

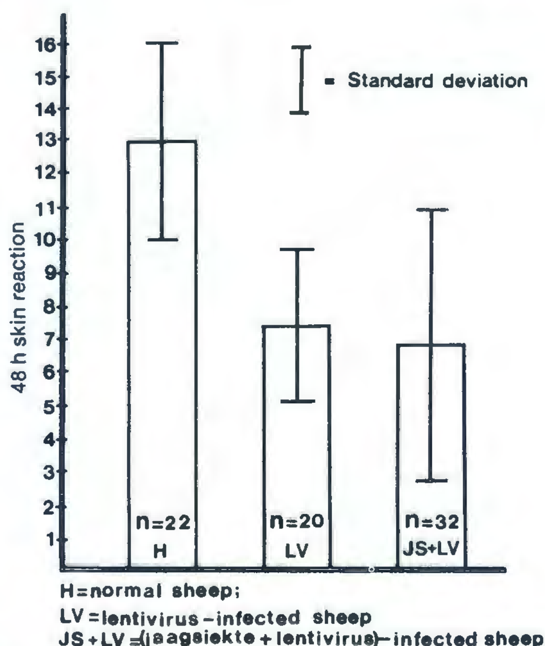

FIG. 2 Tuberculin skin reaction in normal, lentivirus-infected and jaagsiekte plus lentivirus-infected sheep

Monocyte involvement in the immunosuppression

The relatively mild nature of the immunosuppression detected in jaagsiekte-infected sheep suggested a different mechanism from that in AIDS, where inhibition of the T-helper lymphocyte function causes a dramatic inhibition of CMI (Wong, Staal & Gallo, 1985). Since it was known that the sheep lentiviruses have a natural tropism for cells of the macrophage lineage (Narayan *et al.*, 1983), the ability of monocytes, isolated from JS-sheep, to synthesize DNA was compared with the skin test results of the same animals. A linear regression curve relating skin measurements to incorporation of tritiated thymidine (as a measure of DNA synthesis) is shown in Fig. 3. It suggests that reduced metabolic activity of monocytes may be responsible for the depressed cellular immune reaction, indirectly implicating the lentivirus in this process.

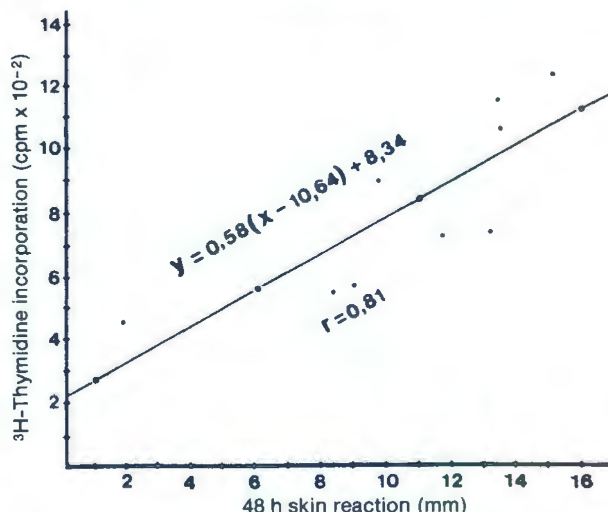

FIG. 3 Regression curve ^{3}H-thymidine incorporation (cpm $\times$ 10^{-2}) vs. skin sensitivity (mm)

The reduced DNA synthesis observed in these blood monocytes also correlated well with the susceptibility of sheep to jaagsiekte. In Table 1 the average cpm-values obtained in ^{3}H-thymidine incorporation studies in a group of JS-sheep are compared with those of normal and resistent sheep. The latter group consisted of animals which were inoculated with a standard dose of JSRV but were free from clinical jaagsiekte symptoms after 12 months.

Decreased monocyte activity therefore correlates with a reduction of the delayed hypersensitivity reaction and with enhanced susceptibility to jaagsiekte.

Effect of lentivirus replication on the chemotactic response of alveolar macrophages (AMs)

Replication of lentivirus has been shown to depend on the level of differentiation/maturation of the monocyte/macrophage (Gendelman, Narayan, Kennedy-Stoskopf, Kennedy, Ghotbi, Clements, Stanley & Pezeshkpour, 1986). In order to assess the effects of lentivirus replication on their chemotactic activity, alveolar macrophages were collected from JS-infected sheep by lung lavage and cultured for 10 days *in vitro*. The clarified supernatants of these cultures were then tested for the presence of virus by means of reverse transcriptase and immunoblot assays as previously described (Payne *et al.*, 1986). Two AM preparations positive for lentivirus production (LV+) and 2 negative preparations (LV−) were then assayed for chemotactic potential in response to N-F Met, a synthetic chemoattractant, as described under MATERIALS AND METHODS. The response of these *in vivo* infected AMs is shown in Fig. 4. Positive chemotaxis was observed in the 2 negative control cultures (C and D), whereas in the two LV(+) cultures (A and B) the response was inhibited and a higher level of chemokinesis (25 % values) was observed.

To confirm that this loss of chemotactivity was caused by the *in vitro* activation of lentivirus replication, the experiment was repeated using AMs from normal, healthy sheep. Cultures of these AMs were subsequently infected *in vitro* with lentivirus. When virus production was detected by the RDP assay after 3 days, the cells were tested for their ability to respond chemotactically to tumour cell supernatant (Myer *et al.*, 1987a). Fig. 5 B

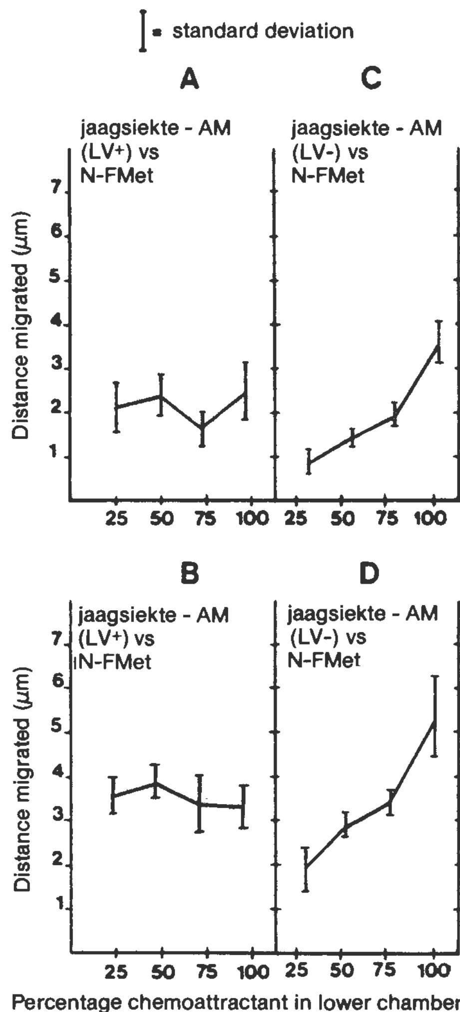

FIG. 4 Effect of lentivirus replication on the chemotactic response of alveolar macrophages infected *in vivo* with lentivirus

illustrates a loss of chemotaxis in infected cells compared to that of normal macrophages (Fig. 5 A).

These results suggest that lentivirus replication in macrophages inhibit their response to a chemotactic stimulus.

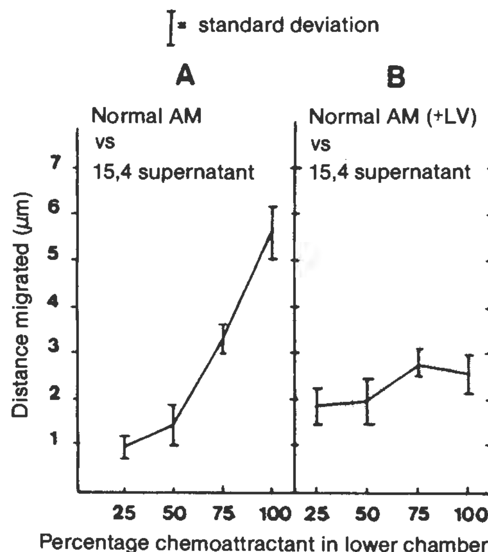

FIG. 5 Effect of lentivirus replication on the chemotactic response of alveolar macrophages infected *in vitro* with lentivirus

TABLE 1 ^{3}H-thymidine incorporation by peripheral blood monocytes from normal, resistant and JS-sheep

Group	Number of animals	Group average cpm	Standard deviation
Normal	4	1115	± 30
Resistant	4	1131	± 102
Jaagsiekte	6	695	± 99

Pasteurella haemolytica infection as a test for immunosuppression

As controls, a group of 9 lambs were sensitized with *M. avium* 1 week after birth. Nine weeks later a tuberculin test was carried out, the results indicting a normal group average skin swelling of 9,0 mm (Table 2), which was lower than the 13 mm obtained for a larger group of adult animals (Fig. 2). When these sheep were inoculated 2 months later with 1×10^{11} *P. haemolytica* organisms, one showed no symptoms at all, 7 had a transient fever reaction only, and one animal died with acute septicaemia.

Seven lambs were infected with 1×10^{6} RDP units of lentivirus within one week after birth and sensitized with *M. avium* 4–9 weeks later. Their delayed hypersensitivity response was measured after 6 weeks. The results show considerable variation between the individual animals, one animal showing a normal skin swelling of 10,4 mm. The average for the others was 5,8 ± 2,3 mm (not shown in Table 2) which is below that of the controls of 9 ± 2,9 mm. A student's t test according to Dunn (1977), between the 9 and 5,8 mm averages, gave a t value of 2,3 (13 degrees of freedom), corresponding to a 97,5 % level of significant difference between the control and lentivirus-infected sheep. By oversight these lambs were immunized with pasteurella vaccine at 2 weeks of age. Nevertheless, when they were challenged at an average age of 7 months, with the same high dose of virulent organisms as the controls, the reactions were significantly stronger (Table 2). Two out of seven animals died from acute septicaemia, and one from pneumonia, while 4 showed clinical signs of respiratory distress (pneumonia) such as coughing dyspnoea and nasal discharge. These 4 sheep recovered clinically, while *P. haemolytica*, reisolated from the lungs of those animals that had died, was subsequently shown to be type 1, thereby confirming that the fatal infection was caused by the organisms inoculated.

DISCUSSION

The human and ovine lentiviruses are similar in morphology and morphogenesis and also share some homologous nucleotide sequences (Gonda, Wong-Staal, Gallo, Clements, Narayan & Gilden, 1985). For both groups the primary target cell seems to be the monocyte/macrophage. In the case of HIV most primary isolates from brain and lung tissues produce 10 to 100-fold more virus in mononuclear phagocytes than in lymphocytes. The virus appears to have a unique ability to acquire an affinity for T4 lymphocytes however, and the prototype HIV, which was selected for growth in T cells, has a 10 000-fold lower ability to infect macrophages than T-cells (Gartner, Markovits, Markovitz, Kaplan, Gallo & Popovic, 1986). At least part of the immunosuppressive effect of HIV is thought to be caused by depletion of T4-lymphocytes.

In contrast visna virus, the prototype ovine lentivirus, does not seem to be able to infect lymphocytes. In addition, virus replication in macrophages is severely restricted *in vivo* (Gendelman, Narayan, Molineaux, Clements & Ghotbi, 1985). This may be the reason why

TABLE 2 Susceptibility of lentivirus-infected sheep to *P. haemolytica* compared with that of normal control animals

Sheep No.	Skin swelling (mm)	Age (weeks) (at challenge)	Symptons		
			Fever	Clinical signs	Post mortem
Controls					
4690/5	8,1	19	Neg	Neg	ND
4685/8	10,3	19	+ (d6)	Neg	ND
4684/1	7,9	19	+ (d4)	Neg	ND
4679/5	6,9	19	–	Died (d4)	Septicaemia pneumonia
4676/1	3,1	19	+ (d4)	Neg	ND
4663/2	9,9	19	+ (d4)	Neg	ND
4659/3	11,8	19	+ (d4)	Neg	ND
4654/4	12,0	20	+ (d6)	Neg	ND
4652/8	11,2	20	+ (d4)	Neg	ND
Average:	9,0				
Standard deviation	2,9				
Lentivirus-infected sheep					
3675/4	7,5	30	+ (d4)	Died (d4)	Septicaemia pneumonia
3672/1	10,4	29	–	Died (d4)	Septicaemia pneumonia
3886/9	2,9	28	+ (d4–6)	++,pneumonia	ND
3930/5	4,3	27	+ (d4)	+++,pneumonia orchitis	ND
3931/3	8,7	27	+ (d4–8)	+++,pneumonia	Pneumonia orchitis
4018/5	4,0	25	++ (d4)	+,pneumonia	ND
4019/3	7,2	25	++ (d4)	+++,died (d7)	pneumonia
Average:	6,43				
Standard deviation	2,8				

ND = not done

immunosuppression has not been observed in maedi-visna and other related infections such as ovine progressive pneumonia (OPP) (Larsen, Hyllseth & Krogrud, 1982; Haase, 1986). Narayan, Sheffer, Clements & Tennekoon (1985) have shown that visna virus causes an interferon-inducing factor to be produced on the surface of infected macrophages which is recognized by lymphocytes of ovine and caprine species. These lymphocytes, in turn, produce an interferon (non-glycosylated, 54–64 kilodalton protein that is resistant to pH 2,0 and heat treatment) that probably restricts viral replication in infected macrophages and is most likely an important mechanism for the persistence of the virus in the host which may develop a chronic immunosuppressive syndrome. In addition, the interferon also results in persistent expression of Ia antigen on the surface of infected macrophages (Kennedy, Narayan, Ghotbi, Hopkins, Gendelman & Clements, 1985).

Using a delayed type hypersensitivity assay, we found a significant immunosuppression in sheep co-infected with lentivirus and jaagsiekte retrovirus. The fact that lentivirus replication is stimulated in jaagsiekte lungs (Dawson *et al.*, 1985) would lead one to speculate that it may also be responsible for the immunosuppression observed, but a direct contribution by the JSRV cannot be excluded in this system. We were able to demonstrate, however, that infection with lentivirus only, will also cause a significant suppression of the delayed type hypersensitivity reaction in sheep.

It was furthermore shown that inhibition of monocyte activity is involved in the suppression, suggesting a primary role for the lentivirus which has the monocyte/macrophage as primary target cell. Further evidence for the involvement of ovine lentiviruses in cell-mediated immunity is the demonstration of decreased lymphocyte-generated interleukin-2 and decreased concanavalin-A-induced suppressor cell activities in natural cases of OPP (Ellis & De Martini, 1985).

Friedlander, Jahrling, Merrill & Tobey (1984) have demonstrated inhibition of mouse peritoneal macrophage DNA synthesis with Arenavirus Pinchinde, while Dengue virus infection causes a sharp decline in their Fc-receptor-mediated attachment and phagocytosis of opsonized sheep erythrocytes (Chaturvedi, Nagar & Mathur, 1983). The pathogenesis of certain viruses is limited to maturation of macrophages as well as to the differentiation of monocytes to macrophages. Lentivirusses have a tropism for cells of the macrophage lineage, and replication of the virus increases as monocytes mature to macrophages (Gendelman *et al.*, 1986). A similar situation was found in our sheep that were infected with lentivirus, and in these animals where *in vitro* replication of the lentivirus was occurring in macrophage cultures, a concomitant inhibition of chemotactic potential was demonstrated in these cells (Fig. 4 & 5).

Insertion of retroviral glycoproteins within infected macrophage membranes is thought to interfere with the Fc-receptor-cytoskeletal system (Marcelletti & Furmanski, 1978). Macrophage function, such as tumour target cell destruction, chemotaxis, phagocytosis and bactericidal activities all require an intact Fc-receptor-cytoskeletal system. Cianciolo, Hunter, Silva & Haskill (1981) reported that a 15 kilodalton protein, isolated from human malignant lymphomas, rendered human monocytes immobile. They were able to neutralize this effect by adding three different murine monoclonal antibodies against p15(E) of type C retroviruses. The bulky, hydrophobic p15(E) protein is thought to block microtubule formation that is required for cell movement (Rojko & Olsen, 1984).

Influenza virus infection of alveolar macrophages reduces their antibacterial efficiency, which is associated with secondary infections in the lungs of infected hosts (Warshauer, Goldstein, Akers, Lippert & Kim, 1977). In these sheep where macrophage chemotaxis might have been inhibited (assuming *in vivo* replication of the lentivirus), secondary complications, such as bacterial pneumonia, could be expected to have led to a more rapid death of the host than would have been caused only by the lung tumour.

Since it has proved impossible to obtain JSRV preparations free from lentivirus, we decided to use an independent non-viral challenge system as an additional test

for immunosuppression. *Pasteurella haemolytica* is a common cause of pneumonia in ruminants but is difficult to induce experimentally in normal animals. Reproducible infection has only been achieved after pre-infection with bovine herpesvirus in calves (Hilwig, Songer & Reggardio, 1985) or *Mycoplasma ovipneumonia* in sheep (Gilmour, Jones, Rae & Quirie, 1986). We have isolated lentivirus from a number of field cases of pneumonic pasteurellosis in sheep (unpublished results), suggesting the possibility that the virus may play a role in the aetiology of this disease. The results reported in this paper support this contention. Despite the fact that the lentivirus-infected lambs were immunized with pasteurellosis vaccine, challenge with a high dose of organisms clearly showed that lentivirus-infected lambs were more susceptible than the controls. The severity of the symptoms in individual animals did not correlate well with the severity of immunosuppression as reflected by decreased skin swelling. Such a correlation can be found, however, when the averages for the groups are compared, suggesting that immunosuppression is the mechanism by which lentivirus enhances infection with *P. haemolytica*. The discrepancy may reflect inconsistencies in the delayed type hypersensitivity assay due to variable responses between individual sheep, or its ability to reflect which lymphoid cell component is involved in immunosuppression.

It can be concluded, therefore, that ovine lentiviruses cause a mild immunosuppression in sheep, mainly by affecting the monocyte/macrophage activity. The suppression of the cellular immune response in turn, predispose the animal to secondary infections such as jaagsiekte and pasteurellosis.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the excellent technical assistance of Erika Stander, Suzette Olivier, S. Drake, Marianne Oosthuizen and Gottfried J. Schiele.

REFERENCES

CARLSON, G. P. & KANEKO, J. J., 1973. Isolation of Leukocytes from bovine peripheral blood. *Proceedings of the Society for Experimental Biology and Medicine*, 142, 853 – 856.

CHATURVEDI, U. G., NAGAR, R. & MATHUR, A., 1983. Effect of dengue virus infection on Fc-receptor functions on mouse macrophages. *Journal of General Virology*, 64, 2399 – 2407.

CIANCIOLO, G., HUNTER, J., SILVA, J. & HASKILL, J. S., 1981. Inhibition of monocyte responses to chemotaxins are present in human cancerous effusions and react with monoclonal antibodies to the p15(E) structural protein of retroviruses. *Journal of Clinical Investigation*, 68, 831 – 844.

DAWSON, M., VENABLES, C. & JENKINS, C. E., 1985. Experimental infection of a natural case of sheep pulmonary adenomatosis with maedi-visna virus. *Veterinary Record*, 116, 588 – 589.

DE MARTINI, J. C., ROSADIO, R. H., SHARP, J. M., RUSSELL, HELENA I. & LAIRMORE, M. D., 1987. Experimental co-induction of type D retrovirus-associated pulmonary carcinoma and lentivirus-associated lymphoid interstitial pneumonia in lambs. *Journal of the National Cancer Institute*, 79, 167 – 177.

DUNN, OLIVE J., 1977. Basic statistics: A primer for the biomedical sciences. 2nd ed pp. 218. New York, London, Sydney & Toronto: John Wiley & Sons, Inc.

ELLIS, J. E. & DE MARTINI, J. C., 1985. Ovine interleukin-2: partial purification and assay in normal sheep with ovine progressive pneumonia. *Veterinary Immunology and Immunopathology*, 8, 15 – 25.

FRIEDLANDER, A. M., JAHRLING, P. B., MERRILL, P. & TOBEY, S., 1984. Inhibition of mouse peritoneal macrophage DNA synthesis by infection with Arenavirus Pinchinde. *Infection and Immunity*, 43, 283 – 288.

GARTNER, SUZANNE, MARKOVITS, P., MARKOVITZ, D. M., KAPLAN, M. H., GALLO, R. C. & POPOVIC, M., 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science*, 233, 215 – 218.

GEBALLE, A. P., VENTURA, P., STOWRING, L. & HAASE, A. T., 1985. Quantitative analysis of visna virus replication *in vivo*. *Virology*, 141, 148 – 154.

GENDELMAN, H. E., NARAYAN, O., MOLINEAUX, S., CLEMENTS, JANICE E. & GHOTBI, Z., 1985. Slow persistent replication of lentiviruses: role of tissue macrophages and macrophage precursors in bone marrow. *Proceedings of the National Academy of Science* USA, 82, 7086.

GENDELMAN, H. E., NARAYAN, O., KENNEDY-STOSKOPF, SUZANNE, KENNEDY, P. G. E., GHOTBI, Z., CLEMENTS, JANICE E., STANLEY, J. & PEZESHKPOUR, G., 1986. Tropism of sheep lentiviruses for monocytes: susceptibility to infection and virus gene expression increase during maturation of monocytes to macrophages. *Journal of Virology*, 58, 67 – 74.

GILMOUR, J. S., JONES, G. E., RAE, A. G. & QUIRIE, M., 1986. Comparison of single strains of four serotypes of *Pasteurella haemolytica* biotype A in experimental pneumonia of sheep. *Research in Veterinary Science*, 40, 136 – 137.

GONDA, M. A., WONG-STAAL, F., GALLO, R., CLEMENTS, JANICE E., NARAYAN, O. & GILDEN, R. V., 1985. Sequence homology and morphologic similarity of HTLV-III and visna virus, a pathogenic lentivirus. *Science*, 227, 173 – 174.

HAASE, A. T., 1986. Pathogenesis of lentivirus infections. *Nature*, 322, 130 – 136.

HAM, R. G., 1965. Clonal growth of mammalian cells in a chemically defined synthetic medium. *Proceedings of the National Academy of Science, U.S.A.*, 53, 288 – 293.

HILWIG, R. W., SONGER, J. G. & REGGARDIO, C., 1985. Experimentally induced pneumonic pasteurellosis: dose-response relationships and protection against natural reinfection in calves. *American Journal of Veterinary Research*, 46, 2585 – 2587.

HOUWERS, D. J. & TERPSTRA, C., 1984. Sheep pulmonary adenomatosis. *Veterinary Record*, 114, p 23.

KENNEDY, P. G. E., NARAYAN, O., GHOTBI, Z., HOPKINS, J., GENDELMAN, H. E. & CLEMENTS, JANICE E., 1985. Persistent expression of Ia antigen and viral genome in visna-maedi virus-induced inflammatory cells. *Journal of Experimental Medicine*, 162, 1970 – 1982.

LARSEN, H. J., HYLLSETH, B. & KROGRUD, J., 1982. Experimental maedi virus infection in sheep: cellular and humoral immune response during three years following intranasal inoculation. *American Journal of Veterinary Research*, 43, 384 – 389.

MARCELLETTI, J. & FURMANSKI, P., 1978. Spontaneous regression of Friend-virus-induced erythroleukemia. *Journal of Immunology*, 121, 1 – 8.

MARKSON, L. M., SPENCE, J. B. & DAWSON, M., 1983. Investigation of a flock heavily infected with maedi-visna virus. *Veterinary Record*, 112, 267 – 271.

MYER, M. S., 1987. The role of the alveolar macrophage in jaagsiekte. Ph.D thesis, University of the Witwatersrand, Johannesburg, South Africa.

MYER, M. S., VERWOERD, D. W. & GARNETT, HELEN, M., 1987a. Production of a macrophage chemotactic factor by cultured jaagsiekte tumour cells. *Onderstepoort Journal of Veterinary Research*, 54, 9 – 15.

MYER, M. S., VERWOERD, D. W. & GARNETT, HELEN M., 1987b. Demonstration of growth-inhibitory as well as growth-stimulatory factors in medium conditioned by lung lavage cells stimulated with a chemotactic factor secreted by jaagsiekte tumour cells. *Onderstepoort Journal of Veterinary Research*, 54, 123 – 130.

NARAYAN, O., KENNEDY-STOSKOPF, S., SHEFFER, D., GRIFFIN, D. E. & CLEMENTS, JANICE E., 1983. Activation of caprine-arthritis-encephalitis virus expression during maturation of monocytes to macrophages. *Infection and Immunity*, 41, 67 – 73.

NARAYAN, O., SHEFFER, D., CLEMENTS, JANICE E. & TENNEKOON, G., 1985. Restricted replication of lentiviruses. Visna viruses induce a unique interferon during interaction between lymphocytes and infected macrophages. *Journal of Experimental Medicine*, 162, 1954 – 1969.

PALSSON, P. A., 1976. Slow virus diseases of animals and man. pp. 17 – 58, Amsterdam: North Holland Publishing Company.

PAYNE, ANNA-LISE, YORK, D. F., DE VILLIERS, ETHEL-MICHELE, VERWOERD, D. W., QUÉRAT, G., BARBAN, V., SAUZE, N. & VIGNE, R., 1986. Isolation and identification of a South African lentivirus from jaagsiekte lungs. *Onderstepoort Journal of Veterinary Research*, 53, 55 – 62.

POPOVIC, M., SARNGADHARAN, M. G., READ, E. & GALLO, R. C., 1984. Detection, isolation and continuous production of cytopathic retrovirus HTLV-III from patients with AIDS and pre-AIDS. *Science*, 224, 497.

QUÉRAT, G., BARBAN, V., SAUZE, N., VIGNE, R., PAYNE, ANNA-LISE, YORK, D. F., DE VILLIERS, ETHEL-MICHELE & VERWOERD, D. W., 1987. Characteristics of a novel lentivirus from South African sheep with pulmonary adenocarcinoma (jaagsiekte). *Virology* 158, 158 – 167.

ROJKO, J. & OLSEN, R. G., 1984. The immunobiology of feline leukemia virus. *Veterinary Immunobiology & Immunopathology*, 6, 107 – 165.

SHARP, J. M., ANGUS, K. W., GRAY, E. W. & SCOTT, F. M. M., 1983. Rapid transmission of sheep pulmonary adenomatosis (jaagsiekte) in young lambs. *Archives of Virology*, 78, 89 – 95.

SHARP, J. M. & HERRING, A. J., 1983. Sheep pulmonary adenomatosis: demonstration of a protein which cross reacts with the major core proteins of Mason-Pfizer monkey virus and mouse mammary tumour virus. *Journal of General Virology*, 64, 2323 – 2327.

SNYDER, S. P., DE MARTINI, J. C., AMEGHINO, E. & CALETTI, E., 1983. Coexistence of pulmonary adenomatosis and progressive pneumonia in sheep in the central sierra of Peru. *American Journal of Veterinary Research*, 44, 1334 – 1338.

VERWOERD, D. W., PAYNE, ANNA-LISE, YORK, D. F. & MYER, M. S., 1983. Isolation and preliminary characterization of jaagsiekte retrovirus (JSRV). *Onderstepoort Journal of Veterinary Research*, 50, 309 – 316.

VERWOERD, D. W., TUSTIN, R. C. & PAYNE, A., 1985. Jaagsiekte: an infectious adenomatosis of sheep. *In:* Pathobiology of viral diseases in domestic animals. Ed. OLSEN, R. C., CRC Press.

VERWOERD, D. W., WILLIAMSON, ANNA-LISE & DE VILLIERS, ETHEL-MICHELE., 1980. Aetiology of jaagsiekte: transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. *Onderstepoort Journal of Veterinary Research*, 47, 275 – 280.

WANDERA, J. G., 1971. Sheep pulmonary adenomatosis. *In:* BRANDLEY, C. A. & CORNELIUS, C. E. (ed.) Advances in veterinary Science and Comparative medicine, 15, 251 – 283. New York & London: Academic Press.

WARSHAUER, D., GOLDSTEIN, E., AKERS, T., LIPPERT, W. & KIM, MARGARET, 1977. Effect of influenza viral infection on the ingestion and killing of bacteria by alveolar macrophages. *American Review of Respiratory Disease*, 115, 269 – 277.