IMMUNOLOGICAL AND VIROLOGICAL INVESTIGATIONS INTO SPORADIC OVINE LYMPHOMA

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ABSTRACT

In New Zealand malignant ovine lymphoma is a low--prevalence, sporadic disease affecting sheep 4 years and older. The aetiology of the disease is unknown but a previous study showed that when cell-free extracts of ovine lymphomas were inoculated into *in utero* and new-born lambs they developed a persistent lymphocytosis and cell-mediated immunity to the lymphoma extracts. Furthermore, particles interpreted as virus-like were observed by electron microscopy in phytohaemagglutinin-stimulated lymphocytes from these sheep. This thesis reports the continued investigation of sporadic ovine lymphoma including its immunological characterisation and a search for evidence of conventional retroviruses.

Techniques for the detection of T cell-specific antigens, surface immunoglobulin, complement receptors and Fc receptors on lymphocytes from the blood and mesenteric lymph nodes from normal sheep were established and ranges of values for T and B cells determined. The use of α naphthyl acetate esterase (ANAE) as a specific marker for sheep T cells was investigated. No correlation was found between T lymphocytes and ANAE-positive cells.

Cells from 17 cases of lymphoma were characterised for the presence of T cell antigens and surface immunoglobulin, markers for T and B cells respectively. Seven cases were T cell and 6 were B cell in origin; in one case cells displayed both markers and in another there were similar numbers of both T and B cells; the cells from the remaining 2 cases had neither marker.

An apparent correlation was found between the patho-anatomical classification of the disease and the immunological origin of the lymphoma. All the T cell lymphomas were of multicentric distribution, whereas 4 of the 6 B cell neoplasms were confined to the alimentary tract and its associated lymphoid tissue. There was no correlation between the cellular morphology of the lymphomas and whether they were T or B cell in origin.

Electron microscopy of several ovine lymphomas and their suspension cultures revealed only a few nonbudding

particles with the dimensions of retroviruses. Smaller vesicular structures were seen associated with cells from a single suspension culture and in control cultures but these were considered unlikely to be of viral origin.

Attempts were made to establish continuous cell cultures derived from ovine lymphomas. These included the culturing of lymphoma cells either over normal fibroblast "feeder" monolayers in various combinations of media and sera, or in simple suspension cultures. Alternatively, lymphoma tissue was used in plasma clot explant cultures. Limited success occurred only with the plasma clot explant cultures where cells from one lymphoma survived 2 passages. Several fibroblast cell lines derived from the lymphoma explant cultures have been developed and passaged over 20 times.

To detect the presence of retroviruses in a variety of materials derived from ovine lymphomas, 2 biochemical techniques were used. The first involved the assay of culture supernatants for ^{3}H uridine-labelled virus in density gradients, and the second the search for RNA-dependent DNA polymerase (RDDP) activity in various preparations. Four ovine lymphoma cell suspension cultures were assayed for the production of RNA-containing virus particles. Tritiated uridine was added at the time the cultures were established and the media harvested after 96 hr. The pellets obtained following differential ultracentrifugation were centrifuged through 15 to 60 percent sucrose gradients and fractions of the gradient were assayed for acid-precipitable radioactivity. Although radiolabelled material was detected at densities of 1.15 to 1.18 gm per ml in preparations of 2 lymphoma cultures, normal lymph node cultures yielded similar results. Radiolabelled material treated with sodium dodecyl sulphate and sedimented through a sucrose gradient had a sedimentation value of approximately 7S. No high molecular weight RNA consistent with that of retroviruses was found. Further experiments using normal ovine fibroblasts led to the conclusion that the radiolabelled material detected in the 15 to 60 percent sucrose gradient was probably slowly sedimenting cellular RNA.

The RDDP assay was performed on ultracentrifuged

preparations from media of lymphoma and normal lymph node cultures, and Rous sarcoma and bovine leukaemia viruses were used as positive controls. Incorporation of ^{3}H thymidine triphosphate into acid-insoluble material was detected in 4 of 16 ovine lymphoma cultures but was also found in material from control lymph node cultures. Little variation in incorporation kinetics could be evinced by altering the assay conditions, and the observed activity was not associated with a particle of density 1.15 gm per ml. Furthermore, RDDP could not be detected in preparations from the homogenates of 6 lymphomas. It was concluded that the activity observed in both the lymphoma and control lymphocyte preparations was not due to RDDP.

Depressed responsiveness by lymphocytes to nonspecific mitogens has been associated with infections by retroviruses in other species. However, there were no differences in responses to phytohaemagglutinin by lymphocytes from lymphoma-inoculated and control sheep.

Although conventional retroviruses have not been clearly demonstrated in association with sporadic ovine lymphoma in these experiments, the failure to detect virus does not rule out the possibility of retroviral involvement at some stage of lymphomagenesis. The development of more sensitive techniques might allow the detection of low levels of virus or viral nucleic acid sequences within cells.

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their understanding and forbearance, but most of all I would thank my wife Roselyn for her love, her support and her willingness to share in all aspects of my life. This thesis is dedicated to her and my parents.

ERRATA

*p.44, line 34: r^2=0.0484 not r^2=0.484

*p.71, line 33: defective not detective

*p.110, line 32: exogenous not endogenous

*p.111, lines 7 to 9: read deoxyribonucleoside triphosphates not ribonucleoside triphosphates

*p.130, line 6: 1.15 not 1.5

*p.130, line 10: gradients not gradinets

*p.132, line 14: were not was

*p.190, line 17: aseptically not asceptically

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ABBREVIATIONS USED IN TEXT

AEV	avian erythroblastosis virus
ALL	acute lymphoblastic leukaemia
ALV	avian leukaemia virus
AMV	avian myeloblastosis virus
ANAE	alpha naphthyl acetate esterase
ATV	antibiotics-trypsin-versene
BLV	bovine leukaemia virus
BLV-FOS	bovine leukaemia virus-infected foetal ovine spleen cells
C	complement
CLL	chronic lymphocytic leukaemia
Con A	Concanavalin A
c src	cellular equivalent of the *src* gene
DMBA	7,12-dimethylbenz(a)anthracene
E	erythrocyte
EA	erythrocyte-antibody complex
EAC	erythrocyte-antibody-complement complex
env	envelope gene of retroviruses
erb	oncogene of avian erythroblastosis virus
ES	equine serum
FBS	foetal bovine serum
FeLV	feline leukaemia virus
FITC	fluorescein isothiocyanate
FOCMA	feline oncornavirus-associated cell-membrane antigen
FOSK	foetal ovine skin cells
^{3}H TTP	tritiated thymidine triphosphate
IgG-latex	IgG-coated latex particles
LTR	long terminal repeat
mac or *myc*	oncogene of MC29 virus
MC29	strain of avian myelocytomatosis virus
MEM	minimum essential medium (Eagle's)
MuLV	murine leukaemia virus
myb	oncogene of avian myeloblastosis virus
OGG	ovine gamma globulin
OLF	ovine lymphoma fibroblasts
OS	ovine serum
PBS	phosphate buffered saline
PHA	phytohaemagglutinin

PRC-CEF	Prague strain Rous sarcoma virus-infected chick embryo fibroblasts
PWM	pokeweed mitogen
RDDP	RNA-directed DNA polymerase
RNAase	ribonuclease
ROK	a diploid foetal ovine kidney cell line
RSV	Rous sarcoma virus
SDS	sodium dodecyl sulphate
SIg	surface immunoglobulin
src	oncogene of Rous sarcoma virus
TCA/NaPP	trichloroacetic acid and sodium pyrophosphate mixture
TdT	terminal deoxynucleotidyl transferase
ZC	complement-coated zymosan particles

PREFACE

The animal lymphomas that are used as research models for human lymphomas and leukemias have, in most cases, a retroviral aetiology (Kaplan, 1978). Although these models provide a framework for human studies, their relevance could be questioned because there is little epidemiological evidence for the horizontal transmission and hence for the involvement of an infectious agent in the human diseases (Kaplan, 1978). Other animal lymphomas such as sporadic bovine lymphoma (Burny *et al.*, 1978; Kettmann *et al.*, 1978), canine lymphoma (Onions, 1980) and a certain proportion of feline lymphomas (Francis *et al.*, 1979) are reported in which retroviruses are not detected as readily as those of the conventional laboratory species. These may be of importance in establishing whether or not retroviruses have a role in the human lymphoproliferative disorders.

In New Zealand, sporadic ovine lymphoma is a low-prevalence lymphoid neoplasm which has not been observed to occur in specific areas or flocks (Johnstone, 1974). As it was of unknown aetiology, Johnstone *et al.* (1979a) attempted to transmit the disease by inoculating *in utero* or new-born lambs with cell-free extracts of the neoplasm. The initial results were encouraging, in that a persistent lymphocytosis developed in 19 of the 28 sheep inoculated. Electron microscopy of phytohaemagglutinin-stimulated lymphocytes from these sheep showed unenveloped particles 80 to 100 nm in diameter which were interpreted as being virus-like by the authors. These particles were seen in large cytoplasmic vacuoles but budding forms were not found. Cell-mediated immunity to cell-free extracts of ovine lymphomas was demonstrated in the sheep showing persistent lymphocytosis and this was considered further evidence in support of a viral aetiology (Johnstone *et al.*, 1979b). However, detectable lymphomas did not develop in any of these animals (Johnstone, unpublished data).

The aim of the experimental work reported in this thesis on sporadic ovine lymphoma was to investigate further this disease both as an entity in itself and as a possible model for lymphomas in man. The research involved immunological

and virological investigations and is reported here in 2 sections. The first of these, Section A, describes the application of immunological and cytochemical methods to the identification of ovine T and B cells. After ascertaining the reliability of these methods with lymphocytes from normal sheep, they were then applied to a number of ovine lymphomas.

The second section, Section B, reports on experiments to clarify whether or not a retrovirus is associated with the disease. In addition to the electron microscopic examination of neoplasms for virus and attempts at establishing long-term suspension cultures, other experimental procedures were applied to enhance the sensitivity of retroviral detection in lymphomas and their culture materials and to provide information about the nature of any particles isolated. These experiments included attempts to detect RNA-containing particles and to identify RNA-directed DNA polymerase in preparations from sporadic ovine lymphomas. Finally, the mitogenic responsiveness of blood lymphocytes from the lymphoma-inoculated sheep of Johnstone *et al.* (1979a) were compared with those of normal sheep as altered responses could be considered indirect evidence for retroviral infection (Dent, 1972).