3. Studies of Cancer in Animals

During the study of natural retroviral infection in non-human primates, it became apparent that many African and Asian non-human primate species had serum antibodies that cross-reacted with HTLV-I antigens. Prevalence of serum antibodies found in these populations varied from < 10 to > 80% and generally increased with age. African green monkeys (*Cercopithecus aethiops*) and macaque species generally had the highest seroprevalence (Miyoshi *et al.*, 1983; Hayami *et al.*, 1984; Ishikawa *et al.*, 1987; Fultz, 1994). A virus isolated from lymphoid cell lines established from seropositive monkeys was shown by Southern blot analysis of genomic DNA, nucleotide sequence analysis and type-specific synthetic peptide epitopes to be 90–95% homologous to HTLV-I (Komuro *et al.*, 1984; Tsujimoto *et al.*, 1985; Watanabe *et al.*, 1985; Ishikawa *et al.*, 1987; Rudolph *et al.*, 1991) and was designated as simian T-cell lymphotropic virus type I (STLV-I).

3.1 HTLV-I in animal models

3.1.1 *Non-human primates*

Six cynomolgus (*Macaca fascicularis*) and two squirrel (*Saimiri sciureus*) monkeys were infected experimentally with HTLV-I by inoculation with autologous lymphoid cell lines immortalized by and producing HTLV-I. To produce the cell lines, monkey peripheral blood mononuclear cells were co-cultivated with lethally irradiated MT-2 cells producing HTLV-I. All the cell lines had monkey karyotypes, grew continuously and expressed IL-2 and virus-specific proteins of HTLV-I. Specific antibodies against HTLV-I and transformed HTLV-I-infected peripheral blood cells were found in the inoculated monkeys. No neoplastic lesion was detected up to two years after inoculation (Nakamura *et al.*, 1986).

Table 6. Case–control studies of the association of HTLV-I infection with malignancies other than adult T-cell leukaemia/lymphoma

Reference	Cancer site		Cases	HTLV-I+ (%)	Controls	HTLV-I+ (%)	Odds ratio	Comments
Asou *et al.* (1986)	All sites except ATLL		394	15.5	Healthy volunteers	3.0	2.2	Excludes cases with history of blood transfusion
	Liver only		33	15.2	Healthy volunteers	3.0	2.6	
Iida *et al.* (1988)	Liver		40	17.5	Local blood donors	4.7	NG	6/7 HTLV-I$^+$ cases had been transfused
Miyazaki *et al.* (1991)	Cervix	< 59 years	88	10.2	Healthy volunteers	[3.3]	2.9	
		> 60 years	65	16.9	Healthy volunteers	10.2	1.7	
	Ovary		37	2.7	Healthy volunteers		0.87	
	Vagina		8	50.0	Healthy volunteers		7.4	
	Endometrium		28	7.1	Healthy volunteers		0.97	
Kamihira *et al.* (1994)	Liver		181	20.4	Local blood donors	3.8	NG	HTLV-I seropositivity was associated with HCV seropositivity
Okayama *et al.* (1995)	Liver		43	30.2	HCV-positive chronic hepatitis	9.5	12.8 (males) 1.3 (females)	Transfusion prevalence similar in cases and controls
Strickler *et al.* (1995)	Cervix		49	14.3	Benign, ASCUS, koilocytotic atypia or CIN I	3.3	3.8	Cases had invasive carcinoma or CIN III

NG, not given; CIN, cervical intraepithelial neoplasia; ASCUS, atypical squamous cells of unknown significance

3.1.2 *Other models*

Adult T-cell leukaemia-like disease was experimentally induced by injection of an HTLV-I-transformed rabbit T-cell line into syngenic rabbits. The cell line was obtained from peripheral blood of a two-month-old virus-infected (B/J × Chbb:HM) F1 rabbit. Fifty per cent of 13 intraperitoneally inoculated newborn rabbits died or were moribund within seven days. Four rabbits surviving for four weeks had detectable cellular cytotoxic activity against transformed cells, increased leukocyte counts and abnormal lymphocytes with convoluted or lobulated nuclei. Histologically, leukaemic infiltrates, probably direct cell-line progeny, were seen in the liver, lung, spleen and mesenteric lymph nodes. The same cell line and dosage killed two syngenic adult rabbits when given intravenously. Three adult virus carriers, 8–15 months of age, were resistant to similar doses (Seto *et al.*, 1988). [The Working Group noted the incomplete description of the lymphoid infiltrates and that this system was not sufficiently characterized to be accepted as a useful model.]

LTR-*tax* transgenic and HTLV-I infected severe combined immunodeficient (SCID) mouse models are discussed in Sections 4.3.2 and 4.3.4 respectively.

3.2 STLV-I in non-human primates

3.2.1 *STLV-I-associated lymphomas* (see also Table 7)

Malignant lymphoma is the most commonly occurring neoplasm in non-human primates and is found most frequently in Old World species (reviewed in Beniashvili, 1989).

Homma *et al.* (1984) detected serum antibodies to membrane antigens of HTLV-I-infected cells in 11 of 13 macaques (*Macaca cyclopis, M. mulatta, M. fascicularis*) with malignant lymphoma or lymphoproliferative disease. In contrast, these antibodies were found in only 7 of 95 healthy macaques of the same colony.

Voevodin *et al.* (1985) reported that, among Sukhumi lymphoma-prone baboons (*Papio hamadryas*), serum antibodies to HTLV-I antigens were found by the indirect immunofluorescence test in 57 of 58 lymphomatous baboons but in only 80 of 177 healthy baboons from the same 'lymphoma-prone' colony. The prevalence of HTLV-I antibodies in baboon populations considered to be 'lymphoma-free' was 5–8%. [The Working Group noted that interpretation of these data was complicated by the introduction of human leukaemic blood into the baboon colony. This blood was not evaluated for viral infection before use (Lapin, 1969). Herpesvirus papio (HVP) was also endemic in the colony.] Later it was shown that monoclonally integrated STLV-I proviral information of rhesus origin was present in the lymphomatous tissue of these baboons (Voevodin *et al.*, 1996).

Srivastava *et al.* (1986) detected antibodies reactive against HTLV-I by several assays, including western blot analysis, in three serum samples collected over a period of approximately four years from a 24-year-old female gorilla (*Gorilla gorilla graueri*) with non-Hodgkin's lymphoma. Morphologically, the neoplasm was diagnosed as a T-cell

Table 7. Lymphoid neoplasia in STLV-I-positive nonhuman primates

Genus, species	Country	No. of animals	Neoplasm	Proviral integration	Comments	Reference
Papio spp.	Georgia	57	Lymphoma	Monoclonal	Rhesus STLV-I$^+$	Voevodin *et al.* (1985, 1996)
Papio spp.	USA	27	Lymphoma (11 with leukaemia)	ND	–	Hubbard *et al.* (1993)
Papio spp.	USA	1	Leukaemia/lymphoma	ND	–	McCarthy *et al.* (1990)
Cercopithecus aethiops	USA	1	Lymphoproliferative disease	Monoclonal	SIV$^+$/STLV-I$^+$	Traina-Dorge *et al.* (1992)
Cercopithecus aethiops	Japan	6	Leukaemia (1) Pre-leukaemic (5)	Monoclonal	STLV-I$^+$	Tsujimoto *et al.* (1987)
Cercopithecus aethiops	Japan	1	Lymphoma	Monoclonal	–	Sakakibara *et al.* (1986)
Cercopithecus aethiops	USA	1	Lymphoma	ND	–	Jayo *et al.* (1990)
Cercocebus atys	USA	3	Lymphocytosis (1) Leukaemia (1) Lymphoma (1)	ND	SIV_{SMM}^+/STLV-I$^+$	McClure *et al.* (1992)
Macaca cyclops *Macaca mulatta* *Macaca fascicularis*	USA	3 5 3	Lymphoproliferative disease	ND	–	Homma *et al.* (1984)
Gorilla gorilla graueri	USA	1	Lymphoma	Monoclonal	–	Srivastava *et al.* (1986)

ND, not detected

histiocytic lymphoma. [The Working Group noted the lack of immunochemical information to confirm the T-cell origin of the neoplasm.] Southern blot analysis of DNA from *Bam*HI-digested neoplastic tissue using a complete HTLV-I genome probe yielded one 10-kb fragment and a 1.05-kb internal fragment common to all HTLV-I isolates. This confirmed that the gorilla was infected with HTLV-I or a closely related virus. The gorilla was also seropositive for cytomegalovirus, Epstein–Barr-like virus and Yaba virus.

Sakakibara *et al.* (1986) reported lymphoma and leukaemia in a wild-caught female green monkey (*Cercopithecus aethiops*) that was very similar to human ATLL. Neoplastic lymphocyte antigens reacted specifically with antibodies to HTLV-I and were $CD2^+$ ($Leu\ 2a^+$), $CD3^-$ ($Leu3a^-$) and negative for surface immunoglobulin.

Spontaneous malignant lymphomas, including 12 cases with leukaemia and lymphoma, were reported in 28 baboons and one African green monkey. All the lymphoma cases were seropositive for HTLV-I antigen, while the prevalence in the 3200-member baboon colony was about 40%. The disease in these monkeys had many similarities to ATLL in humans, including skin involvement, adult onset, generalized lymphadenopathy, hepatosplenomegaly, anaemia, leukaemia, hypercalcaemia, pulmonary involvement and similar histological and immunocytochemical features. Immunohistochemically, 24 of the lymphomas were of T-cell origin, two of B-cell lineage, two could not be identified as B- or T-cell origin and one was not evaluated (Jayo *et al.*, 1990; McCarthy *et al.*, 1990; Hubbard *et al.*, 1993).

McClure *et al.* (1992) found T-cell leukaemia, lymphocytosis and lymphoma, respectively, in three 10–23 year-old sooty mangabeys (*Cercocebus atys*) naturally infected with SIV_{SMM} and STLV-I. Another dual infection of SIV and STLV-I together with a lymphoproliferative disease was observed in an African green monkey (Traina-Dorge *et al.*, 1992).

3.2.2 *Pathological and molecular aspects*

Ishikawa *et al.* (1987) established 11 cell lines of virus-producing lymphoid cells in the presence of IL-2 from five species of STLV-I antibody-positive non-human primates. The cell lines expressed T-cell activation markers and either $CD3^+$ or $CD2^+$, expressed viral antigens that reacted with sera from human ATLL patients and monoclonal antibodies against p19 and p24 of HTLV-I core protein, and produced virus particles with RNA-dependent DNA polymerase activity. DNA from these cell lines contained proviral sequences similar to HTLV-I but with different restriction patterns.

Peripheral blood lymphocyte chromosomal DNA taken from 31 wild-caught captive STLV-I-seropositive African green monkeys was evaluated for proviral integration of STLV-I. One of these monkeys was overtly leukaemic and five were pre-leukaemic. Preleukaemia was diagnosed by finding abnormal lymphocytes in the peripheral blood. The monoclonal integration sites of the proviral genome in these six monkeys indicated proliferation of STLV-I-infected cells. Restriction patterns with *Pst*I and *Sst*I were the same as those for prior isolates from African green monkeys, except that three animals had deletions of one *Pst*I site, suggesting that the virus could be defective in these cases.

Lymphocytes from seropositive monkeys without leukaemic changes did not contain provirus detectable by Southern blot and were polyclonal. The development of ATLL-like disease with monoclonal integration of STLV-I proviral genome indicated that STLV-I has similar leukaemogenicity to HTLV-I (Tsujimoto *et al.*, 1987). [The Working Group noted that the pre-leukaemic diagnosis in five animals was tentative, as abnormal lymphocytes were found in STLV-I-seronegative monkeys, and it is difficult to correlate the occurrence of abnormal lymphocytes with seropositivity.]

Lymphoproliferative disease was diagnosed in an African green monkey with monoclonally integrated STLV-I. STLV-related sequences were identified by Southern blot analysis of DNA extracted from hyperplastic lymphoid tissue. This animal was also infected with SIV and was immunodeficient, as suggested by wasting, cryptosporidial intestinal infection and relatively low levels of $CD4^+$ and high levels of $CD8^+$ lymphocytes (Traina-Dorge *et al.*, 1992). [The Working Group noted that the immunodeficiency diagnosis was questionable.]

Moné *et al.* (1992) established a cell line from a non-Hodgkin's lymphoma of a baboon and detected monoclonally integrated STLV-I proviral DNA, using Southern blot assay and HTLV-I PCR.

An STLV-I rhesus strain (*M. mulatta*) has been characterized in lymphomas from Sukhumi baboons (*Papio hamadryas*). Thirty-seven STLV-I isolates were investigated by PCR which discriminated rhesus-type and baboon-type STLV-I strains. The PCR results were confirmed by DNA sequence data. Partial nucleotide sequences of both STLV-I isolates from lymphomatous baboons were 97–100% homologous to known rhesus STLV-I and 85% homologous to conventional baboon STLV-I. This macaque-to-baboon inter-species transfer of STLV-I may have initiated the outbreak and increased the incidence of lymphoma among Sukhumi baboon colonies (Voevodin *et al.*, 1996).

3.3 Bovine leukaemia virus in sheep and cattle

Bovine leukaemia virus (BLV), HTLV-I, HTLV-II and STLV constitute a unique subgroup within the retrovirus family, characterized by a distinct genetic content, genomic organization and strategy for gene expression (Cann & Chen, 1990; Gallo & Wong-Staal, 1990; Burny *et al.*, 1994; Kettmann *et al.*, 1994). Although BLV is not as closely related to HTLV-I as are the STLVs, much more is known about its pathogenicity and transmission. Therefore the carcinogenicity of BLV is considered here. BLV infection has been eradicated from western European cattle. No evidence of human infection has been documented.

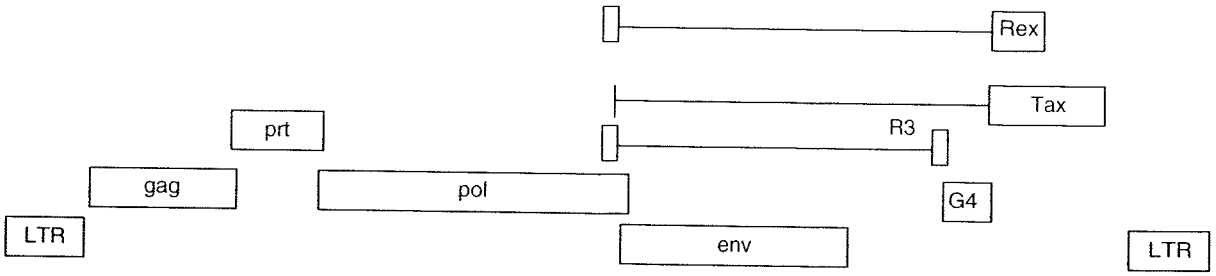
BLV is a transactivating retrovirus recognized as the etiological agent of enzootic bovine leukosis (reviewed in Burny *et al.*, 1994; Kettmann *et al.*, 1994; Schwartz & Lévy, 1994). Presence of the virus has been reported in cattle, sheep, water-buffaloes and capybaras (a South American rodent, *Hydrochoerus hydrochaeris*). Experimental induction of tumours by BLV has been carried out in cattle, sheep and goats (Kettmann *et al.*, 1984), but it is not known if tumours can be induced in water-buffaloes and capybaras.

Replication of these viruses is regulated at the transcriptional and post-transcriptional levels by their own regulatory proteins, notably Tax and Rex. Infection is followed by a long latent period, and only a small proportion of infected individuals develop the terminal neoplastic disease. BLV virions are difficult to identify in neoplastic tissue, but can be found in normal or neoplastic lymphoid cells from BLV-infected cattle or sheep (Jensen *et al.*, 1991; Powers & Radke, 1992). Apart from the difference in host range, a notable difference between BLV and HTLV is that infection by BLV is associated with malignancy of B cells, whereas HTLV affects T cells (Paul *et al.*, 1977).

BLV provirus comprises 8714 bp, making up the following genes (Figure 10):

- *gag*, representing the genetic information for the matrix (p15), capsid (p24) and nucleic acid-binding (p12) proteins;
- *prt*, encoding the viral protease, p14;
- *pol*, the gene for reverse transcriptase and integrase (852 amino acids);
- *env*, the gene for gp51 (268 amino acids) and gp30 (214 amino acids), the external and transmembrane glycoproteins respectively;
- *tax*, the genetic element coding for a transactivator protein, p34 Tax;
- *rex*, the sequence coding for the Rex protein (p18), a molecule involved in the export of genomic RNA from the nucleus;
- R3 and G4, two open reading frames coding for protein products of 44 amino acids and 105 amino acids, respectively, that upregulate BLV expression in the infected host.

Figure 10. Genomic organization of BLV provirus[a]



[a]From Schwarz & Lévy (1994)

Transmission of BLV occurs mainly via transfer of infected lymphocytes by contaminated needles, syringes, etc. Transmission can also occur via milk and *in utero* (Schwartz & Lévy, 1994). Infection can be experimentally transmitted to sheep, goats, pigs, rabbits, monkeys and buffalos (Kettmann *et al.*, 1984; 1994). Tattooing, dehorning, rectal palpation and vaccination procedures can be involved in the transmission of BLV via contaminated blood (Foil & Issel, 1991). Once established, infection is lifelong. The viral load of the inoculum, the time since infection, the efficiency of virus propagation and clonal expansion of BLV-infected cells are key factors determining the number of infected cells at any given time and the probability of neoplastic transformation.

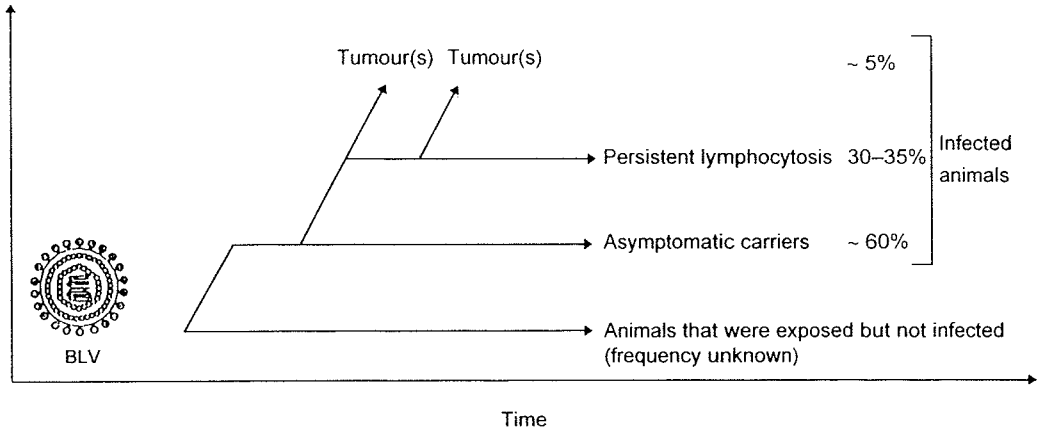
Experimental transmission via biting insects (Foil & Issel, 1991) or intradermal inoculation of BLV proviral DNA into sheep (Willems *et al.*, 1992b; 1993) has been reported.

The presence of BLV within a host is detected by agar-gel immunodiffusion and ELISA. PCR is not useful because viral propagation is slow and the immunogenicity of the virus is high. Seropositivity is detected by gp51 ELISA within two to three weeks after infection (see Kettmann *et al.*, 1994).

3.3.1 *Disorders induced by BLV*

BLV is associated with enzootic bovine leukosis (EBL) (also called bovine leukaemia, bovine lymphoma, bovine lymphosarcoma, bovine malignant lymphoma), which is the most common neoplastic disease of cattle. In terms of the long-term progression of BLV infection, cattle fall into three major groups (see Figure 11). The first and largest of these groups includes those animals (about 60%) that develop a persistent infection and humoral immune response but are normal in every other respect. The second group, representing 30–35% of all BLV-infected cattle, develop persistent lymphocytosis, a disorder that results from polyclonal expansion of the B-lymphocyte population (Kettmann *et al.*, 1980a,b). The third, and much smaller, group (about 5% of infected animals), includes animals that develop leukaemia/lymphosarcoma.

Figure 11. BLV-induced pathogenesis in cattle



From Kettmann *et al.* (1994)

BLV is the etiological agent of not only bovine leukosis but also ovine leukosis. Although less than 5% of BLV-infected cattle go on to develop tumours, all experimentally infected sheep progress to and die in the tumour phase of the disease and after shorter latency periods than cattle (Djilali *et al.*, 1987; Djilali & Parodi, 1989; Gatei *et al.*, 1989).

(*a*) *Cattle*

Three types of EBL are clinically recognized (International Committee on Bovine Leukosis, 1968):

Calf multicentric type: This is characterized by rapidly growing generalized lymph node enlargement with bone marrow involvement. Lymphocytes infiltrate various internal organs, particularly late in the disease.

Adult multicentric type: There is usually lymph node enlargement which may be either symmetrical or asymmetrical. Any tissue in the body may be infiltrated by neoplastic cells and clinical signs depend on the organs or organ systems involved.

Skin leukosis: The first sign may be an urticaria-like change in the skin, especially on the neck, back, rump and thighs. Lymph nodes may be enlarged and the skin lesions may become covered with a thick scab. There may be complete healing of skin lesions and lymph node regression. However, the disease may take a fatal course with typical lymph node involvement and neoplastic-cell infiltration of organs.

(*b*) *Sheep*

The haematological disorders associated with BLV infection are less well defined in sheep. BLV-infected sheep do not develop a persistent lymphocytosis lasting for years, as is seen in cattle. Some infected animals develop lymphosarcoma with no previous haematological disorder (Djilali & Parodi, 1989; Ohshima *et al.*, 1991b). Lymphoid leukaemia and localized lymphosarcoma frequently occur together (Gatei *et al.*, 1989; Ohshima *et al.*, 1991a; Murakami *et al.*, 1994a).

3.3.2 *Pathological and molecular aspects*

(*a*) *Cattle*

BLV persists in peripheral B-lymphocytes (Paul *et al.*, 1977) and the proportion of B-lymphocytes in the peripheral blood of BLV-positive animals increases before any potential increase in the number of circulating lymphocytes (Fossum *et al.*, 1988). Persistent lymphocytosis, when it develops (Figure 11), is a polyclonal expansion of the B-cell population, including BLV-infected and BLV-uninfected cells (Kenyon & Piper, 1977). The ratio of infected to uninfected cells is roughly 1 : 3 to 1 : 4 (Kettmann *et al.*, 1980a). Animals are considered to be in persistent lymphocytosis when successive total lymphocyte counts significantly exceed normal values (International Committee on Bovine Leukosis, 1968).

Studies of the heritability of susceptibility to persistent lymphocytosis led to the conclusion that persistent lymphocytosis is familial (Abt *et al.*, 1970; Lewin & Bernoco, 1986; Lewin *et al.*, 1988). BLV-infected B-cells from cows with persistent lymphocytosis expressed high levels of major histocompatibility complex (MHC) class II, surface IgM and CD5 antigen. Cells expressing CD11b and CD11c, normally expressed by cells of the myeloid lineage were also found. $CD5^+$ cells from BLV-positive cattle, whether with persistent lymphocytosis or not, are activated, cycling cells that respond to IL-2 (Matheise *et al.*, 1992).

In persistent lymphocytosis, proviral DNA is integrated at many genomic sites in BLV-positive circulating leukocytes. In lymphosarcoma, in contrast, proviral DNA is integrated at only one or a few sites. Tumours result from a mono- or oligoclonal proliferation of cells. Integration sites, however, are not conserved from one animal to another. For example, DNAs from 25 independent hamster × bovine somatic-cell hybrids were analysed by Southern blot with probes made of unique cell DNA fragments adjacent to single-copy proviruses from three different bovine tumours. It appeared that these cellular sequences, and thus the respective proviruses, belonged to three different chromosomes in the three tumours examined (Grégoire *et al.*, 1984). No rearrangement of cellular DNA sequences flanking a BLV provirus was found in 28 other BLV-induced tumours (Kettmann *et al.*, 1983). It can be concluded that tumour cells can accommodate proviral DNA sequences at many sites in the genome.

Histological classification of BLV-induced lymphomas was carried out using the National Cancer Institute Working Formulation (Vernau *et al.*, 1992). The distribution of cell types varied much more than in humans. Most of the bovine lymphomas (1067/1198; 89%) were high-grade tumours. The diffuse large-cell type and its cleaved variant comprised 66% of the lymphomas. Follicular tumours were extremely rare (4/1198; 0.3%), in marked contrast to human non-Hodgkin's lymphomas, of which at least 34% are follicular.

Seventeen BLV-induced bovine lymphoid tumours were determined to be of B-cell lineage, based on their immunoglobulin gene rearrangements (Heeney & Valli, 1990). Immunohistochemical studies of bovine lymphosarcomas using a pan-T monoclonal antibody revealed that they all lacked detectable-T cells. Although one tumour failed to react with monoclonal antibodies directed against either T- or B-cell determinants, all others were positive for various B-cell markers. The most frequent phenotype was Ia^+, cytoplasmic IgM^+ or surface IgM^+, with occasional concurrent appearance of the IgG isotype. Cells positive for terminal deoxynucleotidyl transferase (TdT^+) occurred sporadically. It follows that BLV-induced tumours are composed of relatively mature B-cells.

(*b*) *Sheep*

BLV infection in sheep causes increases in circulating B-lymphocytes. Tumours have been described as polymorphic centroblastic lymphosarcoma (Parodi *et al.*, 1982; Parodi, 1987), in which more than 95% of the cells were positive for surface immunoglobulins and MHC class II (Murakami *et al.*, 1994a,b). In one study, coexpression of CD5 and B-cell markers occurred in half of the cases (Dimmock *et al.*, 1990).

Tumours in sheep are monoclonal or oligoclonal expansions of cells carrying proviral information. Most of the tumours tested contained one BLV provirus per genome. In contrast, peripheral blood lymphocytes from aleukaemic sheep and sheep with early lymphocytosis are characterized by polyclonally integrated provirus. Appearance of a clonal subpopulation among cells with polyclonally integrated provirus indicates the onset of leukaemia (Rovnak *et al.*, 1993). Tumours from different sheep harbour the provirus at different sites, suggesting that the mechanisms for tumour initiation are independent of the integration site.

(*c*) *Mechanistic studies*

The mode of cell transformation by BLV remains conjectural. BLV Tax protein probably plays a central role, as it is a major determinant of the replication potential of the virus. The same is true of the protein products of the viral genes R3 and/or G4 (Willems *et al.*, 1994) and of the YXXL motifs of the transmembrane glycoprotein (Willems *et al.*, 1995). Bovine Tax protein complements activated human *ras* p21 in transforming Fischer rat embryo fibroblasts (Willems *et al.*, 1990) and the Tax/*ras* p21 cooperative effect is not hampered by a mutation that abrogates the transactivating activity of Tax protein (Willems *et al.*, 1990; 1992c). It is thus clear that transactivation by Tax and transformation by Tax in collaboration with *ras* p21 are separable functions of the Tax molecule. No data yet demonstrate whether the induction of leukaemia/-lymphoma is affected by cellular oncogenes in cattle, sheep or goats.

Alterations of the *p53* tumour-suppressor gene have been examined in cattle and sheep. No *p53* mutation was found in 10 BLV-induced sheep tumours. In cattle, 5 out of 10 tumours harboured *p53* mutations, whereas only one of seven samples from animals in persistent lymphocytosis showed an alteration of the *p53* gene. It appears that *p53* genomic alterations are not frequently involved in BLV-induced leukaemogenesis in sheep (Dequiedt *et al.*, 1995).

3.3.3 *Vaccination trials*

Protection against retrovirus infection has been achieved in sheep by vaccination with recombinant vaccinia viruses expressing the BLV Env protein (Ohishi *et al.*, 1991; Portetelle *et al.*, 1991). Sheep protected against infection showed a CD4 response to Env peptide 51-70 (Gatei *et al.*, 1993) and a high neutralizing antibody titre (Portetelle *et al.*, 1991). Vaccinated sheep which become infected after challenge with the virus maintain a low viral load for several years without signs of disease.