

3. Studies of Cancer in Animals

3.1 HIV-1 and HIV-2

There have been many unsuccessful attempts to infect a variety of laboratory animal species (rats, hamsters, guinea-pigs) with HIV-1 and HIV-2 (Morrow *et al.*, 1987). In some studies, rabbits have been infected successfully (Filice *et al.*, 1988; Kulaga *et al.*, 1989), but the most reliable models involve HIV infection of nonhuman primates.

Chimpanzees (*Pan troglodytes*) (Morrow *et al.*, 1989), gibbons (*Hylobates lar*) (Lusso *et al.*, 1988) and pigtailed macaques (*Macaca nemestrina*) (Frumkin *et al.*, 1993; Gartner *et al.*, 1994) can be infected with HIV-1, whereas HIV-2 infection has been reported in rhesus monkeys (*M. mulatta*), cynomolgus monkeys (*M. fascicularis*) and baboons (*Papio papio sp.*) (Stahl-Hennig *et al.*, 1990; Castro *et al.*, 1991; Barnett *et al.*, 1994).

Despite persistent infection and immunological disorders such as lymphopenia and a decrease in CD4⁺ T-cell counts, clinical signs are rare in HIV-1- and HIV-2- infected non-human primates. Chimpanzees show definite serological and haematological features of HIV infection (Morrow *et al.*, 1989). No clinical disease was seen in HIV-1-infected pigtailed macaques with persistent HIV-1 infection more than one year after first incubation (Gartner *et al.*, 1994).

Transient lymphadenopathy and/or splenomegaly have been observed in HIV-2-infected rhesus and cynomolgus monkeys (Stahl-Hennig *et al.*, 1990; Livartowski *et al.*, 1992), but in most cases they remained clinically healthy (Putkonen *et al.*, 1989). Diarrhoea and weight loss were reported in one of eight infected rhesus macaques (Castro *et al.*, 1991). A case of central nervous system and lung lesions due to actinomycetes was reported by Livartowski *et al.* (1992).

One rapidly growing mammary adenocarcinoma has been observed in an HTLV-I/HIV-1-infected rabbit (Kulaga *et al.*, 1989). [The Working Group considered that the occurrence of this tumour was probably unrelated to the retroviral infection.]

Among six HIV-2 infected baboons (*Papio cynocephalus*), five animals became persistently infected. After 28 months, one baboon developed an AIDS-like condition with fibromatosis involving lymph nodes, skin, thyroid and pancreas. Another animal was reported to follow a similar clinical course (Barnett *et al.*, 1994).

3.2 Lymphomas in nonhuman primates

Prior to the first documented lymphoma outbreak in colonies of rhesus monkeys, malignant lymphomas in nonhuman primates had been reported only rarely (Stowell *et al.*, 1971). However, lymphomas have been reported to develop in various species of monkeys treated with immunosuppressive agents (Reitz *et al.*, 1980) and in newborn tamarins experimentally infected with Epstein-Barr virus (EBV) (Young *et al.*, 1989). Lymphomas have also been found in various nonhuman primates naturally or experimentally infected with herpesvirus saimiri (HVS) (Adamson *et al.*, 1975), or with STLV-I (see Section 3.2.1 of the monograph on HTLV in this volume, p. 308).

3.2.1 Occurrence of lymphomas in nonhuman primates infected with simian immunodeficiency virus

Lymphomas in simian immunodeficiency virus (SIV)-infected nonhuman primates have been documented in rhesus, cynomolgus and pigtailed macaques, but the incidence of these lymphomas is not well defined. In a study of cynomolgus macaques, an incidence of 38% (9/24) was reported (Feichtinger *et al.*, 1990). In a retrospective necropsy study in the USA, King *et al.* (1983) observed nodular lymphoproliferative infiltrates of well differentiated lymphocytes in liver, kidney and bone marrow tissues in 3/16 macaques (*M. mulatta* and *M. fascicularis*) and, in addition, a clear malignant lymphoma was found in one macaque (*M. mulatta*). All four animals were immunodeficient. Letvin *et al.* (1983) also reported three lymphoma cases in the same colony. [The Working Group noted that it was unclear whether these were the same animals as previously reported.] It was subsequently recognized that this colony was infected with SIV (Letvin & King, 1990).

The likely transfer of nonpathogenic SIV from its natural host (the sooty mangabey monkey: *Cercocebus atys*) to the highly sensitive macaques, as manifested by the development of lymphoma, was demonstrated by Baskin *et al.* (1986). These studies involved the inoculation of a rhesus macaque (*M. mulatta*) with a homogenate of a cutaneous leprosy lesion from a sooty mangabey monkey. Subsequently, the rhesus monkey developed a lymphoma, and cells from this lymphoma induced a further lymphoma when injected into another rhesus macaque. Lymphoblastoid cell lines from the second rhesus macaque were established *in vitro* from tumour cell suspensions and shown to produce a herpesvirus related to EBV and a retrovirus morphologically similar to SIV (Baskin *et al.*, 1986). Baskin *et al.* (1988) also observed one case of lymphoma in a study of 24 rhesus monkeys experimentally infected with this virus designated SIV_{SMM}. SIV_{MNE} was also isolated from a pigtailed macaque (*M. nemestina*) with lymphoma (Benveniste *et al.*, 1986; Henderson *et al.*, 1988). SIV_{MNE} was shown to be related to HIV-2.

Five lymphoma cases out of 49 necropsied stump-tailed macaques (*M. arctoides*) were observed by Lowenstine *et al.* (1992). Among these 49 animals, 75% had pathological lesions compatible with a diagnosis of SIV infection and the SIV-related mortality was 68%. SIV_{STM} was pathogenic for rhesus macaques.

In the UK, Ramsay *et al.* (1991) observed B-cell lymphomas in 2/26 rhesus monkeys infected with SIV_{MAC} over a two-year period. These lymphomas occurred 11.5 and 20

months after infection. In a study of 7 rhesus and 3 cynomolgus monkeys infected with SIV_{MAC} or SIV_{SMM}, one animal developed a lymphoma involving the lumbar spinal cord 11.5 months after the onset of SIV infection (Baskerville *et al.*, 1990).

In a Swedish study, malignant lymphoma was observed in 10/33 wild-caught cynomolgus monkeys 5 to 15 months after intravenous inoculation with SIV_{SMM} (Feichtinger *et al.*, 1990, 1992a,b).

3.2.2 *Pathological and molecular features of lymphoma*

The SIV_{SMM}-associated lymphomas in cynomolgus monkeys were clinically malignant, with visceral metastasis, and were in some cases also observed to develop in testis, brain and spinal cord (Feichtinger *et al.*, 1990; Ramsay *et al.*, 1991; Feichtinger *et al.*, 1992a,b). By histology, the lymphomas were mostly high grade and all those tested were phenotypically B-cell derived. Most showed clonal heavy- and light-chain immunoglobulin restrictions and immunoglobulin gene rearrangements (Feichtinger *et al.*, 1990; Ramsay *et al.*, 1991; Feichtinger *et al.*, 1992a,b; Rezikyan *et al.*, 1995).

No integrated viral genomes were found in lymphoma cells (Feichtinger *et al.*, 1990, 1992a,b). In another study, an SIV-like virus was identified in a lymphoblastoid cell line established from a transmissible lymphoma associated with SIV infection (Baskin *et al.*, 1986).

In a monkey cohort in Sweden, the time to lymphoma development varied from five to 46 months after SIV infection. The lymphomas were all of B-cell origin. DNA analysis of VDJ immunoglobulin genes showed both monoclonal and oligoclonal rearrangements. In some instances, the lymphoma clone was already detectable in lymph nodes soon after SIV infection and before manifestation of clinically apparent lymphoma (Rezikyan *et al.*, 1995). All the lymphomas were associated with an EBV-like B-lymphotropic herpesvirus (HVMF-1) (Feichtinger *et al.*, 1990, 1992a,b; Rezikyan *et al.*, 1995; Li *et al.*, 1993a, 1994), which had 65% DNA homology in exonic regions with EBV (Li *et al.*, 1994).

The SIV_{SMM}-related lymphomas have features very similar to those of the AIDS-related lymphomas in man which are associated with EBV, supporting the hypothesis of an important role of EBV-type viruses in the pathogenesis of such lymphomas.

3.2.3 *Other neoplastic conditions*

Neoplastic conditions other than lymphomas have not been documented as being related to SIV infection, with the possible exception of occasional cases of retroperitoneal fibromatosis. However, retroperitoneal fibromatosis has been seen mostly in macaques infected with the simian immunosuppressive type D retrovirus (SRV-2) (Giddens *et al.*, 1985; Tsai *et al.*, 1995) and in one case of SIV-induced AIDS (Baskerville *et al.*, 1990) (see also Section 4.2.3).

3.2.4 *Cofactors in SIV oncogenesis*

As discussed for AIDS-related malignant lymphoma in humans (Section 2.2.4), the interaction of several oncogenic cofactors at various stages of the lymphomagenic

process has to be considered. These factors can be classified into those inducing: (a) activation, (b) deregulated proliferation and (c) genomic abnormalities in B-cells.

Marked B-cell follicular hyperplasia, seen in early stages of SIV as well as HIV infection (Biberfeld *et al.*, 1985; Chalifoux *et al.*, 1986; Kaaya *et al.*, 1993b), could predispose to B-cell lymphomagenesis. In both SIV and HIV infections, viral antigens appear after infection in hyperplastic follicles on the follicular dendritic cells (FDC). These cells have the foremost antigen-presenting effect on follicular B-cells and are therefore related to the development of the characteristic follicular hyperplasia (Biberfeld *et al.*, 1985; Tenner-Rácz *et al.*, 1986; Kaaya *et al.*, 1993b). With progression of infection, the FDC-antigen-presenting cell-reticulum is destroyed, probably by immunopathological mechanisms and/or viral cytopathic effects (Biberfeld *et al.*, 1985; Stahmer *et al.*, 1996). This leads to the breakdown 'lysis' of follicles, which probably is reflected functionally by the development of impaired immune responses to neoantigens. This follicle 'lysis' may promote the selection of FDC-independent, deregulated autocrine B-cells which during migration through extranodal tissues settle and develop into malignant lymphomas. This extranodal homing is probably promoted by the capacity of AIDS-related malignant lymphomas in humans to produce growth factors (IL-6, IL-10) with possible autocrine functions (Emilie *et al.*, 1992).

A highly deregulated cytokine growth factor homeostasis and the disruption of the antigen-presenting FDC network are thus likely also to play an important role in B-cell activation and proliferation with an increased risk for genomic changes and lymphomagenesis in SIV-infected monkeys (Kaaya *et al.*, 1993b).

Despite the clear association of SIV infection with lymphomagenesis, no evidence yet indicates a direct oncogenic effect of the SIV or HIV genome. However, in-vitro experiments have suggested a transforming effect on 3T3 cells transfected with the SIV PBj₁₄ *nef* gene (Du *et al.*, 1995).

The well recognized oncogenic effects of EBV in certain human lymphomas appear to be mirrored in SIV-infected nonhuman primates. Thus studies have shown a direct transforming/immortalizing effect of the EBV-like HVMF-1 in cynomolgus monkeys associated with SIV-related lymphomas (Li *et al.*, 1994).

3.3 Feline immunodeficiency virus infection in cats

Lentiviral infections of animals other than non-human primates include infections with feline immunodeficiency virus (FIV), bovine immunodeficiency virus, maedi-visna virus, caprine arthritis-encephalitis virus and equine infectious anaemia virus (Coffin, 1992). An association between viral infection and the development of neoplasia, in particular B-cell lymphomas, has been documented only for FIV infections.

FIV was first isolated in 1986 and has become recognized as a common infection in pet cats worldwide (Pedersen *et al.*, 1987). Initial epidemiological studies of a representative sample of the pet cat population in the United Kingdom reported a 19% prevalence of FIV in sick cats, a 6% prevalence in healthy cats and a 21% prevalence among cats in households with more than one cat (Hosie *et al.*, 1989). In studies in the United States, 10–14% of sick cats and 1–4% of healthy cats were FIV-positive (Grindem *et al.*, 1989;

Shelton *et al.*, 1989; Yamamoto *et al.*, 1989; O'Connor *et al.*, 1991). In Japan, infection rates as high as 44% in sick cats and 12% in healthy cats have been recorded (Ishida *et al.*, 1989).

High-grade B-cell neoplasms in association with both naturally acquired and experimentally induced infections have been described. The term 'lymphosarcoma' is used throughout the text to designate tumours of lymphoid lineage. Five cases of lymphosarcoma and one case of a poorly differentiated myeloproliferative disorder are the only tumours that have been documented in association with experimental FIV infections (Yamamoto *et al.*, 1988; English *et al.*, 1994; Poli *et al.*, 1994; Callanan *et al.*, 1996). A broader range of tumours in cats with naturally acquired infections has been described and case reports include lymphosarcomas (Shelton *et al.*, 1990; Hutson *et al.*, 1991; Barr *et al.*, 1993; Callanan *et al.*, 1996), fibrosarcomas (Ishida *et al.*, 1989), myeloproliferative diseases (Ishida *et al.*, 1989; Shelton *et al.*, 1990; Hutson *et al.*, 1991), mast-cell tumours (Shelton *et al.*, 1990; Barr *et al.*, 1993; Terry *et al.*, 1995), cutaneous squamous-cell carcinomas (Hutson *et al.*, 1991; Pedersen & Barlough, 1991), miscellaneous adenomas and carcinomas (Gruffydd-Jones *et al.*, 1988; Hopper *et al.*, 1989) and oligodendrogliomas (Hurtrel *et al.*, 1992).

3.3.1 Occurrence of lymphosarcomas in FIV infection

In natural FIV infection, the majority of clinical and epidemiological studies demonstrate that lymphosarcomas occur in less than 10% of FIV-infected cats (Hopper *et al.*, 1989; Hosie *et al.*, 1989; Ishida *et al.*, 1989; Yamamoto *et al.*, 1989; Shelton *et al.*, 1990). Evaluation of the association between FIV infection and lymphoid malignancies is confounded by concurrent infection with the C-type feline leukaemia virus (FeLV), the most common cause of lymphosarcoma in cats (Hardy, 1981). In a study of 161 cats with leukaemia and/or lymphoma, Shelton *et al.* (1990) performed a stratified analysis controlling for FeLV infection using the Mantel-Haenszel test, which revealed a significant association between FIV infection and leukaemia/lymphoma. The estimated relative risk for developing leukaemia/lymphoma was 5.0 for cats infected with FIV only, compared with uninfected cats. In the same study, a relative risk of 62.1 was found for FeLV-infected animals and, when animals were co-infected with both viruses, the risk was 77.3.

Two reports have described lymphosarcomas in two of seven experimentally infected cats at 9 and 21 months after infection (English *et al.*, 1994) and in two of 20 experimentally infected cats at 30 and 42 months after infection (Callanan *et al.*, 1996); the specific pathogen-free cats were infected intravenously or intraperitoneally with the North Carolina State University (NCSU1) or Glasgow (Gla-8) strains of FIV, respectively. Lymphosarcoma associated with experimental infection has also been documented in a cat intravenously infected with FIV (Pisa M2 strain), 18 months after infection (Poli *et al.*, 1994) and a myeloproliferative disorder was reported 8.5 weeks after inoculation with FIV (Petaluma strain) (Yamamoto *et al.*, 1988).

3.3.2 *Pathological and molecular features of lymphosarcoma*

In FIV-associated lymphosarcomas, as with HIV and SIV, sites of tumour distribution are predominantly extranodal, with involvement of the heart, eyes, brain, spinal cord, pancreas and urinary bladder (Hutson *et al.*, 1991; Callanan *et al.*, 1996).

Limited information is available on the immune function of FIV-infected cats with lymphosarcomas. Callanan *et al.* (1992) found normal responses of lymphocytes to mitogens in one case, and Poli *et al.* (1994) detected a marked reduction in circulating CD4⁺ T-lymphocytes in another case.

In a series of eight FIV-infected cats (two experimental and six natural) with lymphosarcoma, seven of the tumours were high-grade B-cell lymphomas of the centroblastic or immunoblastic subtypes. The remaining case was a T-cell tumour associated with concurrent FeLV infection (Callanan *et al.*, 1996). Lymphosarcomas in experimental infection described by English *et al.* (1994) and Poli *et al.* (1994) were also of B-cell origin, based on immunoglobulin expression. However, the single neoplasm described by Poli *et al.* (1994) was low-grade.

Four of the tumours reported by Callanan *et al.* (1996) were examined with molecular probes to establish tumour cell lineage and to screen for integrated viral sequences (Terry *et al.*, 1995). Confirmation of a B-cell origin was supported by the identification of monoclonal or oligoclonal immunoglobulin heavy-chain gene rearrangements and the lack of rearrangements of T-cell receptor β -chain genes in all four cases. Rearrangement of the *c-myc* locus, which occurs in many FeLV lymphosarcomas, was not found in any of the FIV-associated tumours and none of the tumours showed evidence of integrated FIV sequences by Southern blot hybridization. Poli *et al.* (1994) identified DNA of the FIV *gag* gene in many tissues including tumour tissue of an experimentally FIV-infected cat. However, in this tumour tissue, it could not be determined whether the infection was of neoplastic cells.

Thus lymphosarcomas in FIV-infected cats share similar morphological, immunophenotypic and molecular qualities to those associated with HIV and SIV infections. The evidence available supports an indirect role for FIV in tumour development. FIV induces activation of lymphoid tissue, polyclonal B-cell activation and increased serum cytokine levels, all of which may facilitate malignant transformation of B cells (Lawrence *et al.*, 1992; Rideout *et al.*, 1992; Callanan *et al.*, 1993; Flynn *et al.*, 1994).