4. Other Data Relevant to an Evaluation of Carcinogenesis and its Mechanisms

4.1 Immunity and cancer

In mice and humans with inherited or acquired immunodeficiency, only certain types of malignancy are significantly increased in incidence (Weiss, 1993b). Many of these tumours are associated with viruses that have established persistent infections, and others are tumours arising within the immune system. Consideration of malignancies deve-

loping in cases of immunodeficiency caused by factors other than HIV is restricted in this monograph to humans. The highest relative risks in human non-AIDS immunodeficiency are for non-Hodgkin's lymphoma, Kaposi's sarcoma and non-melanoma skin cancer (Beral, 1991b).

4.1.1 *Types of cancer seen in non-HIV-associated human immunodeficiency*

The vast majority of data concerning the incidence of malignancies occurring in persons with an acquired immunodeficiency other than those with HIV infection comes from patient populations undergoing organ transplantation. In addition to immunosuppressive therapy and the foreign graft, such patients are exposed to incidental infections of donor origin. Birkeland *et al.* (1995) reported on the subsequent risk of malignancy in all 5692 renal transplant patients during 1964–86 within the Nordic countries, using the national population-based cancer registries for long-term follow-up. The data were analysed by standardized incidence ratios (SIR), using the population rates as the reference. Overall, there was a significant increase in overall cancer rates of 4.5 for women and 4.6 for men. Very highly increased risks (SIR, ≥ 10-fold) were seen for cancers of the lip, kidney, cervix and vulva–vagina and non-melanoma cancer of the skin and for non-Hodgkin's lymphoma. In addition, there were significantly increased SIRs (2–5-fold) for a range of common malignancies including cancers of the colon, larynx, lung, bladder, prostate and testis. However, only two cases of Kaposi's sarcoma were reported.

Penn (1993) analysed data on a series of 7192 organ transplant patients followed by the Cincinnati Transplant Tumor Registry in the United States up to 1993. [The institutional sources of these patients were not specified.] Only the numbers of subsequent cancer cases were reported; these were compared with the proportional distribution of site-specific malignancy in the 'general population' without statistical analysis. [It is not clear whether the referent distribution was corrected for age and sex.] The most common tumours in the transplant patients were cancers of the skin (predominantly squamous-cell carcinoma) and lip and non-Hodgkin's lymphoma. There were 307 cases (2.4%) of Kaposi's sarcoma. Other common sites included vulva/peritoneum and kidney. The proportion of cervix cancer cases [3.5%] was reported to be the same as that in the general population. Subsequently, Penn and Porat (1995) reported on cases of central nervous system non-Hodgkin's lymphoma in this registry. Of a total of 1332 non-Hodgkin's lymphoma cases recorded, 289 (22%) involved the central nervous system. Penn (1994) similarly reported on the 326 paediatric patients recorded in the Cincinnati Registry. [These patients appear to be also included in the report above.] Compared with the distribution of cancer sites in adult transplant patients, paediatric patients had a higher frequency of lymphoma (50% versus 15%) and a lower proportion of cancers of the skin and lip (20% versus 38%).

Kinlen *et al.* (1979) reported on the follow-up of 3823 renal transplant patients in Australia, New Zealand and the United Kingdom. Compared with age- and sex-specific national mortality rates, the relative risk for any malignancy was 3.5 and that for non-Hodgkin's lymphoma was almost 60, with an excess evident for squamous skin cancer and mesenchymal tumours including one Kaposi's sarcoma.

In a hospital-based series from London, United Kingdom, Gaya *et al.* (1995) reported on 274 renal transplant patients whose graft survived three years or more, using survival analysis and comparison with national rates. Skin cancers were most common, particularly among men, followed by lymphomas and renal, urinary bladder and bronchial cancer. The actuarial risk of development of any tumour was 18.4% at 10 years and 49.6% at 20 years. There was a higher risk among males than among females, which was attributable to a higher incidence of skin cancer.

Schmidt *et al.* (1995) reported on the occurrence of genito-urinary malignancies among 868 renal transplant patients in a hospital-based series in Germany. Twelve cases were noted, of which one was transplanted in the graft. The 11 de-novo cases included four kidney, three cervical and one each of testicular, vulvar, urinary bladder and renal duct carcinomas.

Levy *et al.* (1993b) reported on 556 liver transplant patients followed between 1985 and 1991 at Baylor University in Dallas, TX, United States. Of these, 25 developed new malignancies, including 10 with lymphoma and 9 with at least one skin cancer. Other malignancies seen included lung, breast, prostate, pancreas, hepatocellular and colon cancers and Kaposi's sarcoma.

Dresdale *et al.* (1993) reported on 112 cardiac transplant patients seen at a hospital in Detroit, MI, United States, between 1985 and 1991. Of these, nine developed a new malignancy, including four cancers of the skin, two of the colon, and one each of the bone and bladder and one Kaposi's sarcoma. Guettier *et al.* (1992) reported on 174 cardiac transplant patients from a hospital in Paris between 1984 and 1990. The only malignancies reported were four gastrointestinal non-Hodgkin's lymphomas. Zahger *et al.* (1993) reported two cases of Kaposi's sarcoma occurring among 18 cardiac transplant patients in Jerusalem; both patients were Mediterranean Jews.

Table 29 summarizes the data from more than 15 000 organ (mostly kidney) transplant patients. The most common findings are the substantial excesses of squamous-cell carcinoma of the skin and non-Hodgkin's lymphoma. In addition, risks for cancers of the kidney and urinary bladder, cervix and vulva, and head and neck were commonly increased. Less frequently seen are unusual tumours including Kaposi's sarcoma and testicular cancer.

Table 30 summarizes the experience of more than 11 000 patients receiving bone marrow transplants, primarily for haematopoietic malignancies and disorders. The findings in this patient population were similar to those in the organ transplant patients, with the additional common finding of leukaemia. Kolb *et al.* (1992) reported only the number of new malignancies occurring among the 9732 bone marrow transplant patients reported to the International Bone Marrow Registry and among the 226 patients reported to the European Bone Marrow–European Late Effects Project. Of the former, 116 had a subsequent cancer: 58 were lymphoma, 15 leukaemias including myelodysplasia, 14 cancers of the skin including 5 melanomas, 4 cervical including dysplasia, 3 vulvar/-vaginal, 2 oropharyngeal, 2 breast and 2 thyroid cancers, among others. Among the latter group of patients, there were 11 new cancers including 6 skin cancers.

Table 29. Cancer risks following organ transplants: cohort studies

Reference	Population, number	Time period	Case identification	Comparison group	Sites	Results			Notes
						SIR			
						M	(Σ)	F	
Birkeland *et al.* (1995)	Nordic countries: kidney transplant only 5692 (32 392 person-years) Follow-up for life	1964–86	Population registries	Population rate	Lip	14.0		117.0	SIR increase seen within first 5 years, peaking in the next decade with some decrease after 15 years. Risk higher in younger patients (< 45) and if the donor was a family member. Cyclosporin and OKT3 not used. (All SIRs listed are statistically significant.)
					Colon	3.2		3.9	
					Rectum	4.5		–	
					Larynx	3.8		15.0	
					Lung	1.8		4.9	
					Cervix	–		8.6	
					Vulva/vagina	–		31.0	
					Prostate	2.1		–	
					Testis	3.9		–	
					Ureter/kidney	4.6		19.0	
					Urinary bladder	3.1		17.0	
					Non-melanoma skin	29.0		18.0	
					Brain, etc.	3.0			
					Thyroid	16.0		5.1	
					Connective tissue	7.3			
					NHL	10.0		11.0	
					Hodgkin's lymphoma	–		11.0	
Gaya *et al.* (1995)	Hammersmith Hospital, London — Graft survived 3 years: kidney transplant only 274 (2622 person-years) 29-year follow-up	1961–90	Hospital follow-up	Population rates	Skin	23.3		12.0	4-fold RR increase for non-skin cancers seen within first 5 years and stabilized 2–3 thereafter. RR for skin cancer increased with time. Greater risk in younger patients (< 40 years). No effect seen for cyclosporin. Median time to diagnosis, 7.7 years.
					NHL		(45.0)		
					Kidney	34.0	25.0		
					Urinary bladder	9.5	(7.6)		

Table 29 (contd)

Reference	Population, number	Time period	Case identification	Comparison group	Sites	Results	Notes
Schmidt *et al.* (1995)	University of Cologne: kidney transplant only 868 (1209 person-years) Follow-up 42 ± 45 months	1968–94	Hospital follow-up	Population rates	Genito-urinary cancer only: sites 6 Kidney, renal duct 3 Cervical 1 Testis 1 Bladder 1 Vulva		RR for males, 7.3; females, 11.2 All but one cancer developed in the 324 patients aged 20-40 years [$p = 0.001$]
Penn (1993)	Cincinnati Transplant Tumor Registry 6798	[1968]–93	Special registry	General population		Proportional incidence	
					Skin	52% *vs* 32%[a]	Mean time to diagnosis: KS, 22 months (2–226); lymphomas, 32 months (1–254); epithelial excl. vulva and perineum, 69 months (1–299); vulva, perineum, 113 months (3–286); 94% lymphomas were NHL. In heart or heart–lung transplant cases, 42% were cardiac lymphomas. Large increase in SCC.
					Lymphoma	23% *vs* 5%	
					Lip	7% *vs* 0.3%	
					KS	6% *vs* < 0.1%	
					Kidney	5% *vs* 2%	
					Vulva/perineum	4% *vs* 0.5%	
					Cervix	3% *vs* 3%	
					Hepatobiliary	2.6% *vs* 1.4%	
					Other sarcomas	1.7% *vs* 0.5%	
Penn (1994)	Cincinnati Transplant Tumor Registry: paediatric patients 326	1968–93	Special registry	Adult transplant patients	Lymphoma	50% *vs* 15%	Mean time to diagnosis: KS, 46 months (4–197); lymphoma, 20 months (1–177); skin and lip, 118 months (10–282); vulva/perineum, 140 months (43–262). 98% lymphomas were NHL — these were much more frequent in non-renal transplants. There were six cases of cervix cancer (including in situ) among the 158 females: mean age at diagnosis, 25 years.
					Skin and lip	20% *vs* 38%	
					Malignant melanoma	15% *vs* 5%[b]	
					Vulva/perineum	4% *vs* 3%	
					KS[c]	2% *vs* 4%	
					Other sarcoma	3% *vs* 1%	
					Liver	3% *vs* 2%	
					Thyroid	3% *vs* 1%	
					Cervix	2% *vs* 4%	

Table 29 (contd)

Reference	Population, number	Time period	Case identification	Comparison group	Sites	Results		Notes
						RR	No.	
Kinlen *et al.* (1979)	United Kingdom Australasian Transplant Study 3823	1970–77/8	Special registry	Population rates	NHL Skin[d] Other	58.6 4.5 1.7	34 5 30	(Other: Kidney/bladder, 6; colon, 4; lung, 3; genital, 3; leukaemia, 3; other, 11)
						CI		
Levy *et al.* (1993b)	Baylor University Medical Center: liver transplant only 556	1985–91	Hospital follow-up	NG	Lymphomas Skin	1.7% 1.6%		Mean time to diagnosis: lymphomas, 7 months; skin, 18 months. For skin, ratio of BCC to SCC, 1:4.
Dresdale *et al.* (1993)	Henry Ford Hospital, Detroit: cardiac transplant treated with antilymphocyte globulin 112	1985–91	Hospital follow-up	None	SCC Colon Other	[3%] [2%] [4%]		
Guettier *et al.* (1992)	Hôpital Broussais, Paris: cardiac transplant 174	1984–90	Hospital follow-up	None	NHL	3%		All were gastrointestinal

SIR, standardized incidence rate; OKT3, orthotopic kidney transplantation therapy; NHL, non-Hodgkin's lymphoma; KS, Kaposi's sarcoma; RR, relative risk; NG, not given; CI, cumulative incidence; BCC, basal cell carcinoma of the skin; SCC, squamous cell carcinoma of skin

[a] Proportion of all malignancies

[b] Proportion of all skin cancers

[c] Two of these KS patients were HIV-positive

[d] United Kingdom only

Table 30. Cancer risks following bone marrow transplants: cohort studies

Reference	Population number	Time period	Case identification	Comparison group	Results	Notes
Lowsky *et al.* (1994)	Princess Margaret Hospital, Toronto 557 (1608 person-years)	1970–93	Hospital follow-up	Population rates	Any cancer, relative risk = 4.2 (10 malignancies in 9 patients) 2 oral cavity, 1 malignant melanoma, 2 skin, 1 endometrium, 1 breast, 1 NHL, 1 AML (donor cells), 1 lung. 7 patients developed in situ cancer: 5 cervical, 1 vulvar, 1 rectal Addendum: 1 endometrium, 1 NHL	Risk associated with total body irradiation and development of acute GVHD
Socié *et al.* (1993)	European Bone Marrow Transplantation–Severe Anaplastic Anemia Working Group 748	1971–92	Hospital follow-up	Population rates	Any cancer, relative risk = 28.6 (9 malignancies) 2 acute leukaemia, 5 head and neck, 1 stomach, 1 liver	Rish higher among males, increased with age, and with use of radiation-based conditioning regimen
Socié *et al.* (1991)	Hôpital Saint-Louis, Paris; Fanconi anaemia patients 40	1976–90	Hospital follow-up	Population rates	1 tongue cancer	

Table 30 (contd)

Reference	Population number	Time period	Case identification	Comparison group	Results	Notes
Kolb *et al.* (1992)	International Bone Marrow Transplant Registry: cancer patients 9732				(116 malignancies) 58 lymphoma, 15 leukaemia including myelodysplasia, 14 skin including 5 melanoma, 4 cervical including dysplasia, 3 vulva/vaginal, 12 other solid, 10 unspecified	
	Late Effect Study Group 226				(11 malignancies) 4 within 6 years: 2 squamous cell of skin, 1 breast, 1 chloroma; 7 > 10 years: 4 basal cell of skin, 1 'spinalioma', 1 parotid, 1 uterus	

GVHD, graft versus hot disease; AML, acute myeloid leukaemia; NHL, non-Hodgkin's lymphoma

Lowsky *et al.* (1994) reported on 557 consecutive bone marrow transplant patients from a hospital in Toronto, Canada between 1970 and 1993. The actuarial probability of having a new malignancy was 12% at 11 years after the transplant for the first nine cancers reported. Of the total of 11 patients who developed cancer, three had developed cancer of the skin, two of the oral cavity, two of the endometrium (of whom one also had breast cancer), two had myelogenous leukaemias (one of donor origin), and one each had non-Hodgkin's lymphoma and cancer of the lung.

Socié *et al.* (1993) reported on the experience of 748 patients followed by the European Bone Marrow Transplantation–Severe Aplastic Anemia Working group from 1971 to 1991. [The Working Group noted that these patients may overlap with those of Kolb *et al.* (1992) noted above.] Of these, 748 were treated by bone marrow transplantation. Of the latter, all but 20 (3%) received short-term immunosuppression (primary cyclophosphamide) as a conditioning regimen before transplantation. Nine patients developed a new malignancy, including five cancers of the head and neck, two acute leukaemias, one stomach and one liver cancer. In the group receiving only immunosuppression, 28 myelodysplasias and 15 acute leukaemias were diagnosed, plus 3 liver, 2 breast, and 1 each of stomach and head/neck cancer and non-Hodgkin's lymphoma. The cumulative incidence at 10 years for any secondary malignancy was much higher in the immunosuppressed group (18.8%) than in the bone marrow transplant group (3.1%). In another report, Socié *et al.* (1991) reported on 40 patients who received a bone marrow transplant to treat Fanconi anaemia. [The Working Group noted that these patients may also overlap with those reported by Kolb *et al.* (1992) noted above.] Of these, one boy developed a cancer of the tongue 74 months after the transplantation.

Mueller and Pizzo (1995) reviewed reports on cancers in children with primary immunodeficiencies (Table 31). In these conditions, the occurrence of malignancy is substantial (5–25%) over a variable number of years and is mostly lymphoma, followed by leukaemia. An earlier review by Kinlen (1992) noted that about half of these malignancies were non-Hodgkin's lymphoma, 13% leukaemia and 9% Hodgkin's disease.

4.1.2 *Time of onset of cancers in non-HIV-associated immunodeficiency*

Among the 5692 renal transplant recipients followed on average for 5.7 years reported by Birkeland *et al.* (1995) (see Section 4.1.1), the risk for cancer at all sites was increased nearly four-fold in the first five years, over five-fold in the next decade and four-fold in the subsequent period. The risk for skin cancers increased continuously with time since receiving the transplant. In the registry-based series of 7668 tumours in 7192 patients reported by Penn (1993), the 307 Kaposi's sarcomas appeared on average at 22 (range, 1–226) months after organ transplantation; the 1252 lymphomas at 32 (1–254) months and other tumours at 67 (1–299) months. The average time of onset of non-Hodgkin's lymphoma involving the central nervous system was the same as that seen for all non-Hodgkin's lymphoma: 33 (0.1–249) months (Penn & Porat, 1995). In the paediatric patients from this cohort, the range of time intervals for any malignancy was the same as that for adults. However, the average time of onset of the 8 Kaposi's

Table 31. Cancers arising in children with primary immunodeficiency

Syndrome	Malignancy	Cumulative incidence (%)	Estimated latency in years
X-linked gamma globulinaemia	Leukaemia, NHL	6	10
Wiskott–Aldrich	NHL, leukaemia, Hodgkin's disease	>10	6
Bloom's syndrome	Leukaemia, NHL, Hodgkin's disease, adenocarcinoma	25	During first 40 years
Ataxia telangiectasia	Leukaemia, NHL, Hodgkin's disease, other	>12	9
Common variable immunodeficiency	NHL, stomach	8–10	16
Severe combined immunodeficiency	NHL	5	< 1
X-linked lympho-proliferative	NHL	4	(following EBV infection)
Selective IgA deficiency	NHL, gastric, thymoma	NG	NG

Modified from Mueller & Pizzo (1995)
NHL, non-Hodgkin lymphoma; EBV, Epstein-Barr virus; NG, not given

sarcomas was only 13 months (0–34) and that for the 167 lymphomas was 22 (0.2–217) months (Penn, 1994). In the United Kingdom–Australasian study of 3823 renal transplant patients, the authors noted that the risk for any malignancy was elevated within the first two years, and remained so through ≥ 4 years of follow-up. In the series of 274 renal transplant patients followed on average for 9.6 years reported by Gaya *et al.* (1995), the relative risk was also about four-fold within the first five years, and remained within the same range thereafter. Among non-skin tumours, there did not appear to be a time trend. However, the appearance of skin cancer increased significantly with time. Among the 868 renal transplant patients followed on average for 41.8 months for genito-urinary system malignancies, the 11 cancers (excluding the transplanted kidney adenocarcinoma) occurred on average at 66 (24–131) months (Schmidt *et al.*, 1995).

Fewer follow-up data are available for other organ transplants. In patients who generally receive more immunosuppression, malignancies occur earlier. Among the 556 liver transplant patients followed on average for 35 months by Levy *et al.* (1993b), the 10 lymphomas occurred on average at 8 (1–29) months after transplantation, 1 Kaposi's sarcoma at 16 months, 6 other solid tumours at 34 (12–66) months and 9 skin cancers at 17 (2–66) months. Among the 112 cardiac transplant recipients followed on average for 41.5 months by Dresdale *et al.* (1993), there were one patient with Kaposi's sarcoma at 47 months, four with skin cancer at an average of 43 (8–70) months and four others at 23 (6–60) months. Guettier *et al.* (1992) reported that the four gastrointestinal tract non-

Hodgkin's lymphomas occurring in a cohort of 174 cardiac transplant patients had an average time of onset of 22 (15–29) months. The two cases of Kaposi's sarcoma in cardiac transplant patients in Israel occurred two months after transplantation (Zahger *et al.*, 1993). Among 9732 bone marrow recipients, who generally receive both radiation and chemotherapy, Kolb *et al.* (1992) reported that most of the new malignancies found occurred 'in the first few months', although 9% of 79 patients developed malignancies after more than 10 years of follow-up.

In 1608 patients treated with either immunosuppression or bone marrow transplantation for aplastic anaemia reported by Socié *et al.* (1993) after mean follow-up times of 30 and 47 months, respectively, the median time to development of myelodysplasia syndrome was 52 (2–122) months, that for acute leukaemia was 47 (7–115) months, that for non-Hodgkin's lymphoma was 33 months (one case) and that for other tumours was 52 (1–94) months.

Of 557 bone marrow transplant patients followed by Lowsky *et al.* (1994), a non-Hodgkin's lymphoma developed at 7 months, a leukaemia at 46 months, three skin cancers at an average of 47 (30–64) months and five other cancers at 84 (31–127) months.

Among the cases of congenital immunodeficiency reviewed by Mueller and Pizzo (1995), the length of time to cancer diagnosis ranged from an average of less than one year in severe combined immunodeficiency syndrome to over 40 years in Bloom's syndrome.

4.1.3 *Similarities and differences between AIDS- and transplantation-associated tumours*

(*a*) *In immunity*

HIV-associated immunodeficiency shares with the other acquired or inherited immunodeficiencies reviewed above a diminution of host cellular immunity, the primary control mechanism of latent viral infections. The effect of cyclosporin A, which has been causally associated with an increased incidence of both non-Hodgkin's lymphoma and Kaposi's sarcoma in organ transplant patients, is quite similar to that seen in HIV-infection, with the selective inhibition of T-helper function (IARC, 1990). The populations reviewed above were immunosuppressed by a variety of means, either by inborn genetic defect, by cytotoxic chemotherapy or, in the vast majority of cases, by exposure to a range of therapeutic agents designed to create tolerance to a foreign organ or tissue. In the latter case, the level of immunosuppression can be modulated or withdrawn in response to clinical status, and there is regression of a lymphoma and of Kaposi's sarcoma with reduction or cessation of the treatment (IARC, 1990; Penn, 1993). Further, the impact on the immune system is generally immediate, unlike the apparently cumulative effect that is seen in the natural history of HIV infection. A general characteristic of malignancy occurring in non-HIV/AIDS-related immunosuppression is that the risk and rapidity of onset are directly related to the severity of the immunosuppression (Brusamolino *et al.* 1989; IARC, 1990; Kinlen, 1992; Gaya *et al.*, 1995).

(*b*) *In cancer types*

The types of malignancy which develop excessively in non-HIV-infected patients are generally similar to those seen in AIDS, with a predominance of non-Hodgkin's lymphomas, of which a high proportion involves the central nervous system, and Kaposi's sarcoma. A much higher proportion of non-Hodgkin's lymphomas (> 90%) in transplant recipients are EBV-positive than in AIDS-related non-Hodgkin's lymphoma (~ 50%). Among non-Hodgkin's lymphomas, Burkitt's lymphoma is relatively frequent in AIDS patients and in inherited ataxia telangiectasia and X-linked lymphoproliferative disease (Duncan's syndrome), but rare in adult transplant recipients. Further, in both AIDS and transplant patients, the malignancies tend to be more aggressive and include sites other than those usually seen in the general population (Bayley *et al.*, 1985; Kinlen, 1992; Barrett *et al.*, 1993). In regions where Kaposi's sarcoma in non-immunocompromised patients is relatively frequent, it occurs in transplant recipients at a higher frequency than non-Hodgkin's lymphoma (Qunibi *et al.*, 1988).

Transplant patients also differ from those with AIDS in their excessive development of cancers of the skin, primarily squamous-cell but also basal-cell — particularly with long-term follow-up. In renal transplant patients, there is commonly an excess of cancers of the urinary tract; however, an excess of these cancers has been seen in patients with chronic renal failure without transplantation. In patients treated for haematopoietic diseases, there is an excess of leukaemias; however, this is part of the spectrum of disease seen in many of these conditions. It used to be supposed that the excess cancer risk in transplant patients did not include those fatal malignancies which are common in older non-immunocompromised populations in the developed countries (Kinlen, 1992), and Prehn (1994) postulated that immune reactions may exert a stimulatory effect on such tumours. However, a report from the Nordic countries (Birkeland *et al.*, 1995), consolidating population-based registry data for 5692 renal transplant patients linked to the generally mutually standardized population cancer registries, found, in contrast to other studies, significantly increased risks for the incidence of cancers of the colon, lung, testis, thyroid and prostate.

(*c*) *In onset*

New malignancies in non-HIV-infected immunosuppressed individuals can occur within a very short period. A substantially increased relative risk is consistently seen in the first five years. This is in contrast to the extended latent period preceding the diagnosis of the malignancies seen in HIV-1 infection. The time from start of immunosuppressive therapy to tumour development is shorter in patient groups with more severe immunosuppression. In general, the relative risk for the associated tumours remains fairly constant over time since initiation of treatment, although Kaposi's sarcoma tends to occur earlier than non-Hodgkin's lymphoma; however, the relative risk for skin cancer shows a marked increase with time. In those studies in which both Kaposi's sarcoma and non-Hodgkin's lymphoma were seen, the former generally occurred earlier than the latter, as is seen in AIDS.

4.1.4 *Occurrence of other viruses in malignancies associated with non-HIV immunosuppression*

HHV-8 has been detected in 11/11 biopsies of Kaposi's sarcoma in transplant patients (Boshoff *et al.*, 1995a; Lebbé *et al.*, 1995; Buonaguro *et al.*, 1996). EBV was detected in 28/29 non-Hodgkin's lymphomas from transplant patients (Ho *et al.*, 1985b; Shapiro *et al.*, 1988; Nakhlen *et al.*, 1991). Transplantation of PBMCs from EBV-positive healthy humans into severe combined immunodeficient (SCID) mice frequently results in the development of immunoblastic lymphoma in the immunodeficient mouse.

IARC (1995) reviewed the data on the role of HPV in malignancies among transplant patients. In the case–control studies, the prevalence of cervical infections with HPV detected in women with organ transplants ranged from 22 to 45%, which was significantly higher than that in controls (3–6%). [The Working Group noted that these studies preceded the introduction of more sensitive primers for PCR detection of the high-risk HPV types and probably underestimated HPV prevalence.]

IARC (1995) also reviewed the prevalence of detectable HPV in skin cancers occurring in transplant patients. For 539 squamous-cell carcinoma specimens tested using the more sensitive methods, the positivity rate ranged between studies from zero to 100%, with half of the 16 studies having case positivity rates of at least 50%. Similarly, in eight published studies, among a total of 40 basal-cell carcinomas in transplant patients, nine cases (23%) were scored HPV-positive. A study using new PCR primers detected a high frequency of HPV-5, HPV-8 and other strains related to those occurring in epidermodysplasia verruciformis in skin cancer of renal transplant recipients (Berkhout *et al.*, 1995).

4.1.5 *Mechanisms by which immune dysfunction may contribute to the genesis of cancer*

(*a*) *Activation of oncogenic viruses with immunosuppression*

In immunocompetent persons, cell-mediated immunity may act to limit viral oncogenesis at two levels: first by controlling the overall viral burden by eliminating cells productively infected by the virus; second by recognizing viral antigen expressed on latently preneoplastic and neoplastic cells.

(*b*) *Stimulation and hyperreactivity of remaining cells in immunosuppressed persons*

The presence of the graft itself may modulate the immune system as a source of chronic antigenic stimulation. Lowsky *et al.* (1994) reported that the risk for new malignancies in bone marrow transplant patients was significantly associated with the presence of acute graft versus host disease, but not with the treatment modality itself. Bouwes Bavinck *et al.* (1991) observed that HLA-B mismatching (as well as homozygosity for HLA-DR) was significantly associated with the risk for squamous-cell carcinoma of the skin in renal transplant patients. This association appeared to be independent of the amount and type of treatment. However, B-cell hyperplasia is not a feature of

iatrogenic immunosuppression as it is in HIV infection, which may explain why a larger proportion of non-Hodgkin's lymphomas in AIDS are EBV-negative (see Section 4.3.2).

4.2 Kaposi's sarcoma

Epidemiological and clinical studies (summarized in Section 2.1) have yielded the following conclusions regarding the etiology of Kaposi's sarcoma in HIV-infected individuals:

(i) the immunosuppressive effect of HIV is a major factor;

(ii) HIV component(s) may directly promote the development of Kaposi's sarcoma lesions, as the disease is often more aggressive in HIV-infected patients;

(iii) an infectious agent distinct from HIV and mainly transmitted sexually may have an important role.

This section reviews the virological and cell biological evidence which is relevant to these observations.

4.2.1 *Cell biology of Kaposi's sarcoma lesions*

(*a*) *Origins of Kaposi's sarcoma spindle cells*

The hallmark of the advanced Kaposi's sarcoma lesion is the spindle cell surrounding slit-like spaces. Endothelial cells (either vascular or lymphatic endothelium), cells from venous lymphatic junctions, fibroblasts, smooth muscle cells and dermal dendrocytes have all been proposed as possible progenitors of Kaposi's sarcoma spindle cells (reviewed by Roth *et al.*, 1992; Stürzl *et al.*, 1992a; Kaaya *et al.*, 1995). Rappersberger *et al.* (1991) reported that spindle cells stain with the monoclonal antibody EN-4 (which detects both vascular and lymphatic endothelium) but lack reactivity with the monoclonal antibody Pal-E (which reacts with blood-vessel but not lymphatic endothelial cells). This observation is compatible with spindle cells originating from lymphatic endothelium. However, other markers for blood vessel endothelium (but not lymphatic endothelium; OKM-5 and anti-factor VIII-related antigen; von Willebrand factor; vWF) stain Kaposi's sarcoma endothelial or spindle cells, although slightly varying results have been reported by different laboratories (Nadji *et al.*, 1981; Modlin *et al.*, 1983a; Little *et al.*, 1986; Rappersberger *et al.*, 1991; further references in Roth *et al.*, 1992).

Ultrastructural examination has failed to show the presence of Weibel–Palade bodies, the storage vesicles for vWF and therefore a characteristic feature of vascular endothelium, in spindle cells from Kaposi's sarcoma lesions (Rappersberger *et al.*, 1991). Staining with monoclonal antibody BMA 120, that detects an antigen specific to endothelial cells, lends support to an endothelial origin of Kaposi's sarcoma cells (Roth *et al.*, 1988). Kaposi's sarcoma spindle cells and endothelia lining vascular spaces in lesions express leukocyte adhesion molecule-1 (LAM-1) and thrombomodulin, which are markers of lymphokine-activated endothelial cells (Zhang *et al.*, 1994). This observation supports the notion that Kaposi's sarcoma spindle cells are of endothelial origin and are activated by growth factors (see below).

The staining (observed by some laboratories but not by others) of spindle cells with antibodies to CD14, CD68 and factor XIIIa has been interpreted to reflect a possible link between Kaposi's sarcoma spindle cells and cells of the monocyte/macrophage lineage, possibly dermal dendrocytes (Nickoloff *et al.*, 1989; Rappersberger *et al.*, 1991; Kaaya *et al.*, 1995). These cells are distinct from Langerhans' cells (Nickoloff *et al.*, 1989). The staining of cultured Kaposi's sarcoma spindle cells with an antibody to smooth muscle α-actin (Weich *et al.*, 1991) and other similar histochemical data have been interpreted to suggest a relationship with smooth muscle cells or myofibroblasts (reviewed by Roth *et al.*, 1992). These discrepant results suggest either that cells of different lineages can adopt a spindle-like morphology or that these markers are common to different cells of mesenchymal origin. [The Working Group considered that the weight of evidence pointed to the spindle cells being most closely related to vascular endothelial cells.]

A number of laboratories have cultured cells from Kaposi's sarcoma that express markers characteristic for vascular or lymphatic endothelium (Delli-Bovi *et al.*, 1986; Nakamura *et al.*, 1988; Roth *et al.*, 1988; Siegal *et al.*, 1990; Corbeil *et al.*, 1991; Herndier *et al.*, 1994a), but cultures expressing smooth muscle α-actin (Albini *et al.*, 1988; Wittek *et al.*, 1991) as well as mixed populations (Siegal *et al.*, 1990; further references in Roth *et al.*, 1992) have also been reported. The lineage identity of cultured cells has been defined by staining for the same markers as in the in-situ studies, notably vimentin and cytokeratin (for discrimination of mesenchymal and epithelial cells respectively), the endothelial markers vWF, Pal-E, OKM-5, BMA 120 (specific for blood-vessel endothelium), and EN-4 and UEA-I lectin (reactive with blood-vessel and lymphatic endothelium), CD14, CD68 and factor XIIIa (for the monocyte/macrophage lineage), SMC α-actin (smooth muscle and myofibroblast) and others (reviewed by Roth *et al.*, 1992; Stürzl *et al.*, 1992a; Kaaya *et al.*, 1995). Spindle-shaped cells showing a moderate expression of endothelial antigens have been cultured from peripheral blood of Kaposi's sarcoma patients (Browning *et al.*, 1994).

(*b*) *Vascular lesions induced by Kaposi's sarcoma cell cultures in nude mice*

The various cell cultures established from Kaposi's sarcoma lesions differ in their ability to induce the growth of Kaposi's sarcoma-like vascular lesions in nude mice. A cell line expressing endothelial markers induced Kaposi's sarcoma-like tumours of human origin in nude mice (Siegal *et al.*, 1990; Herndier *et al.*, 1994a). This cell line had a normal diploid karyotype and expressed the endothelial markers factor VIII, EN-4 and UEA-I lectin. In addition, it produced high levels of urokinase plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1; Herndier *et al.*, 1994a). Plasminogen activator has been shown to be involved in the development of endothelial tumours in mice transgenic for the polyoma middle T protein (Montesano *et al.*, 1990). More recently, a second cell line capable of causing tumours of human origin in nude mice has been described and these lesions could be inhibited by β-human chorionic gonadotropin (β-HCG) (Lunardi-Iskander *et al.*, 1995a). These cell lines meet the criteria for a tumorigenic cell line.

In contrast, a few other Kaposi's sarcoma cell cultures, also of an endothelial phenotype, are angiogenic *in vivo*, and induce transient Kaposi's sarcoma-like vascular lesions

of *murine* origin, when inoculated into nude mice (Nakamura *et al.*, 1988; Salahuddin *et al.*, 1988). Spindle-shaped cells grown from the peripheral blood of Kaposi's sarcoma patients have also been reported to induce murine angiogenesis in nude mice (Browning *et al.*, 1994). This angiogenic property, together with other in-vitro findings (see below), suggests that growth factors produced by the cultured cells could induce murine cells to produce lesions resembling early Kaposi's sarcoma.

However, most other cell cultures established from Kaposi's sarcoma lesions, including some which are capable of acidic low-density lipoprotein uptake and expressing the endothelial marker BMA 120 (Roth *et al.*, 1988), did not induce tumour formation in nude mice, were not capable of growing in soft agar and showed only a slightly reduced serum dependence. Similarly, cultures expressing the endothelial marker OKM-5 were not tumorigenic in nude mice (Delli-Bovi *et al.*, 1986).

Cell cultures of smooth muscle origin do not induce Kaposi's sarcoma-like lesions *in vivo* but are capable of local invasion in muscle organ cultures and through artificial basal membranes (Albini *et al.*, 1988; Wittek *et al.*, 1991). The reason for these differences is not clear but may be linked to differences in the cytokine profile secreted by these different cultures (see below).

(c) *Growth factors involved in the proliferation of spindle cells*

Extensive work by several laboratories has examined the role that lymphokines might play during the development of Kaposi's sarcoma. However, probably because of the different cell types grown by different laboratories, the findings reported are inconsistent. Fibroblast growth factors (FGFs) and platelet-derived growth factors (PDGFs) have been found to be expressed in Kaposi's sarcomas, or to be present in short-term cultures from Kaposi's sarcoma biopsies.

(i) *Fibroblast growth factors*

Basic fibroblast growth factor (bFGF) has been reported to be secreted by Kaposi's sarcoma cultures expressing endothelial cell markers and may promote the growth of these cells *in vitro* (Ensoli *et al.*, 1989). Other groups, working with Kaposi's sarcoma cultures of either an endothelial phenotype (Corbeil *et al.*, 1991) or mixed fibroblastoid/-endothelial appearance (Werner *et al.*, 1989) also found an FGF-like activity in supernatants of their Kaposi's sarcoma cultures which stimulated the growth of normal fibroblasts and endothelial cells.

Members of the FGF family, including bFGF and endothelial cell growth factor (ECFG), are known to stimulate the growth of normal endothelial cells, and cultured Kaposi's sarcoma cells with endothelial characteristics have been shown to induce transient neoangiogenesis in nude mice (Nakamura *et al.*, 1988). The FGF family of cytokines may thus play a crucial role during the development of Kaposi's sarcoma. In Kaposi's sarcoma, the expression of bFGF and FGF5 in spindle cells has been shown by in situ hybridization (Xerri *et al.*, 1991). Acidic FGF and FGF6 are also expressed in Kaposi's sarcoma (Li *et al.*, 1993b), but the technique employed in this study (RT-PCR) does not permit the identification of the cell type(s) secreting these two members of the FGF family. The importance of bFGF in the development of experimental Kaposi's

sarcoma-like lesions is further supported by the report that a bFGF-specific antisense oligonucleotide can inhibit the angiogenic effect of cultured Kaposi's sarcoma cells in nude mice (Ensoli *et al.*, 1994a).

(ii) *Platelet-derived growth factor*

Normal endothelial cells (Ensoli *et al.*, 1989; Roth *et al.*, 1989) as well as short-term cultures of endothelial cells with endothelial characteristics (Ensoli *et al.*, 1989) produce PDGF. Kaposi's sarcoma cell cultures that produce PDGF thus do not require exogenous PDGF to promote proliferation (Ensoli *et al.*, 1989; Corbeil *et al.*, 1991). However, PDGF has been found to be essential for the propagation *in vitro* of Kaposi's sarcoma cells expressing the endothelial cell marker BMA 120 and capable of acidic low-density lipoprotein uptake but exhibiting fibroblast-like growth properties. These cultures were also shown to express mRNA for the receptors for PDGF-A and PDGF-B (Roth *et al.*, 1989; Werner *et al.*, 1990). Kaposi's sarcoma spindle cells express *in vivo* mRNA for PDGF-B receptor, whereas mRNAs for PDGF-A and PDGF-B were expressed on some tumour cells located in the vicinity of slit-like spaces (Stürzl *et al.*, 1992b). Taken together, these findings suggest that Kaposi's sarcoma cells related to endothelial cells produce PDGF which is required for the growth of spindle cells exhibiting at least some fibroblastoid characteristics, thus highlighting the interdependence of the different cell lineages found in Kaposi's sarcomas.

(*d*) *Clonality of Kaposi's sarcoma and chromosomal abnormalities*

Individual nodules of HIV-associated Kaposi's sarcoma may contain predominant clonal populations (Rabkin *et al.*, 1995b). It is unknown whether different Kaposi's sarcomas from the same patient contain the same or different clonal populations. Therefore, whether individual lesions are derived from the same (as in a metastatic lesion) or different clones is also unknown. A tumorigenic cell line established from a Kaposi's sarcoma was reported to contain a marker chromosome (Lunardi-Iskandar *et al.*, 1995b). [The Working Group noted that evidence for chromosomal anomalies in primary Kaposi's sarcoma tissue is lacking.] Some short-term cultures of Kaposi's sarcoma biopsies have been noted to contain chromosomal rearrangements, but no consistent pattern has been confirmed either in primary sporadic tumours (Ottolenghi *et al.*, 1974; Scappaticci *et al.*, 1986) or in AIDS-associated tumours (Delli-Bovi *et al.*, 1986; Alonso *et al.*, 1987; Saikevych *et al.*, 1988).

Thus, clonal populations may develop in Kaposi's sarcoma and give rise to monoclonal tumorigenic cell lines.

4.2.2 *The role of HIV-1 Tat in the development of Kaposi's sarcoma lesions*

Experimental evidence suggests that the Tat protein of HIV-1 can enhance the growth of cultured 'endothelial' Kaposi's sarcoma cells (Ensoli *et al.*, 1990; Barillari *et al.*, 1993). In this in-vitro model, Tat is thought to cooperate with bFGF to enhance Kaposi's sarcoma cell proliferation. The effect of Tat seems to be mediated by its binding to a5 a1 and aV a3 integrins via an RGD (i.e. arginine-glycine-aspartic acid) sequence element in

a manner similar to, and replaceable by, their physiological ligands fibronectin and vitronectin (Barillari *et al.*, 1993; Ensoli *et al.*, 1994b).

Several cytokines, including tumour necrosis factor (TNF), interleukin (IL)-1 and γ-interferon, can render normal endothelial and smooth muscle cells susceptible to the growth-promoting effect of Tat (Barillari *et al.*, 1992), possibly by increasing the expression of integrin receptors which interact with Tat (Barillari *et al.*, 1993; Ensoli *et al.*, 1994b). Injection of Tat into nude mice (Ensoli *et al.*, 1994b) or immunocompetent C57/Bl mice (after incorporation into Matrigel; Albini *et al.*, 1994) induces angiogenesis and this effect is potentiated by bFGF (Ensoli *et al.*, 1994b) or heparin (Albini *et al.*, 1994). The formation of Kaposi's sarcoma-like lesions induced by Tat and heparin can be inhibited by the matrix metalloproteinase inhibitor TIMP-2 (Albini *et al.*, 1994) and Tat and bFGF act synergistically to increase the expression of collagenase IV in nude mice (Ensoli *et al.*, 1994b). These studies suggest the involvement of tissue proteinases in the development of Kaposi's sarcoma.

Several groups have investigated the role of HIV-1 *tat* in Kaposi's sarcoma pathogenesis using transgenic mice. Vogel *et al.* (1988) reported the emergence of Kaposi's sarcoma-like lesions in mice transgenic for HIV-1 *tat*. Transgenic mice carrying the early region of BK virus, included in an LTR-*tat* construct, also develop Kaposi's sarcoma-like lesions in addition to other malignancies (Corallini *et al.*, 1993) and extracellular Tat protein released by tumour cell lines derived from these animals protects them from apoptosis under conditions of serum starvation (Campioni *et al.*, 1995). The growth-promoting effect of extracellular Tat on cultured Kaposi's sarcoma cells and endothelial cells (Ensoli *et al.*, 1990; Barillari *et al.*, 1992) suggests that infection by HIV-1 of cells not directly involved in the Kaposi's sarcoma lesion may be sufficient for triggering the sequence of events leading to the development of Kaposi's sarcoma. In keeping with this interpretation, in *tat*-transgenic mice which did develop Kaposi's sarcoma-like lesions, the expression of *tat* was found not in spindle cells but in neighbouring keratinocytes (Vogel *et al.*, 1988). However, other lines of transgenic mice, carrying the complete HIV-1 genome, failed to develop similar lesions (Leonard *et al.*, 1988).

With regard to the question of whether sufficient levels of HIV-1 Tat are present in AIDS-related Kaposi's sarcoma lesions to achieve an angiogenic effect, Ensoli *et al.* (1994b) claimed that HIV-1 Tat could be detected on spindle cells by histochemical techniques. They suggested that Tat originated from a few HIV-1-infected mononuclear cells infiltrating these lesions.

Thus, the ability of Tat, in concert with other growth factors, to induce vascular lesions resembling Kaposi's sarcoma has been documented in a variety of experimental systems. However, this property may not be unique to HIV-1 infection, as supernatants from T-cell lines infected with HTLV-II have been shown to induce the propagation of Kaposi's sarcoma-derived cells *in vitro*. The lymphokine responsible for this growth-enhancing effect has been identified as oncostatin M (Nakamura *et al.*, 1988; Miles *et al.*, 1992; Nair *et al.*, 1992). This suggests that infection by other human retroviruses can lead to the production of lymphokines which promote the growth of cells found in Kaposi's sarcomas. Since some non-human retroviruses have been shown to induce

Kaposi's sarcoma-like lesions in several animal models (see Section 4.2.3), and since mice transgenic for the middle T gene of polyomavirus develop endothelial cell tumours (Bautch *et al.*, 1987), it is conceivable that various microorganisms could initiate such a cascade of events.

4.2.3 *An infectious agent as a cause of Kaposi's sarcoma*

Extensive epidemiological studies, reviewed in Section 2.1, suggest the involvement in the pathogenesis of Kaposi's sarcoma of an agent which can be transmitted sexually, although not exclusively so.

There is no convincing evidence to associate cytomegalovirus, HHV-6, papillomaviruses, hepatitis B virus (IARC, 1994), *Mycoplasma fermentans* or *M. penetrans* with Kaposi's sarcoma.

In the last two decades, several laboratories have either observed or tried to isolate viruses from Kaposi's sarcomas. Giraldo *et al.* (1972) reported the presence of herpes-like viruses in short-term cultures from Kaposi's sarcoma biopsies. The identity of these particles has never been satisfactorily established. Occasional herpes viral particles have also been seen in Kaposi's sarcoma tissue sections (Walter *et al.*, 1984).

C-Type retroviruses were detected in Kaposi's sarcoma biopsies from a group of HIV-negative Kaposi's sarcoma patients from a distinct region of the southern Peloponnese in Greece (Rappersberger *et al.*, 1991). Some of the clinical features of the disease in this group of patients (involvement of oral and genital mucosa and gastrointestinal tract; extensive involvement of facial skin) were reminiscent of African or AIDS-associated Kaposi's sarcoma rather than 'classical' Kaposi's sarcoma. Retroviral particles have also been found in Kaposi's sarcoma biopsies from patients with AIDS (Gyorkey *et al.*, 1984; Schenk, 1986). It is possible that these particles represented HIV-1.

As discussed in Section 3.2.3, there is no really good animal model for Kaposi's sarcoma. However, several animal models have provided indirect evidence supporting a possible role of retroviruses in the pathogenesis of Kaposi's sarcoma. Macaque monkeys infected with the D-type simian retrovirus type 2 (SRV-2) develop retroperitoneal and subcutaneous fibrosis with progressive fibrovascular proliferation, reminiscent of Kaposi's sarcoma lesions (Tsai *et al.*, 1995). Cell cultures established from these lesions induced self-limited, transient spindle cell proliferation, accompanied by pronounced vascularization, when inoculated into nude mice. In fowl, some strains of avian leukosis virus can induce, in addition to lymphoma, disseminated haemangiomatosis characterized by a progression from early patch-like lesions with predominant endothelial cell proliferation to haemangiosarcoma (Victor & Jarplid, 1988). In BALB/c mice, a strain of Moloney murine sarcoma virus (MMSV 349), containing the *mos* oncogene, induces lesions that resemble human Kaposi's sarcoma on the basis of both histopathology and electron microscopy. The *mos* oncogene does not seem to be sufficient to induce these lesions, as another strain of MMSV, also containing the *mos* oncogene, does not induce similar lesions (Stoica *et al.*, 1990).

In addition to some HIV-1 *tat* transgenic mice which develop Kaposi's sarcoma-like lesions (see Section 4.2.2), mice transgenic for the middle T antigen of polyomavirus develop endothelial tumours (Bautch *et al.*, 1987). These reports indicate that a variety of infectious agents or their proteins can induce vascular proliferation which bears some resemblance to Kaposi's sarcoma lesions. Yet it is difficult to extrapolate from these animal models to a candidate for an infectious agent involved in the pathogenesis of human Kaposi's sarcoma.

4.2.4 *The role of human herpesvirus 8*

A new human γ-herpesvirus (HHV-8), also termed Kaposi's sarcoma herpesvirus (KSHV), has been discovered in AIDS-associated Kaposi's sarcoma biopsies (Chang *et al.*, 1994) and is a strong candidate for the 'Kaposi's sarcoma agent' (see Section 2.1.5).

(*a*) *Genomic organization and relationship to other primate herpesviruses*

HHV-8 belongs to the γ_2 subgroup of herpesviruses and is most closely related to herpesvirus saimiri, a T-lymphotropic herpesvirus with transforming potential, found in squirrel monkeys (*Saimiri sciureus*) (Moore *et al.*, 1996). Several of the HHV-8 structural genes show significant levels of sequence homology to the corresponding genes of herpesvirus saimiri, but also to those of the slightly more distantly related EBV, and the organization of a 20 kb central segment of the HHV-8 genome is highly similar to that of these other two γ-herpesviruses (Moore *et al.*, 1996). In addition, HHV-8 contains a homologue of the human *cyclin D* gene and a member of the family of six-protein coupled receptors (Cesarman *et al.*, 1995). The HHV-8 cyclin homologue has been shown to be active in abrogating the function of the retinoblastoma tumour-suppressor protein and could thus be involved in dysregulating cellular proliferation or differentiation.

(*b*) *In-vivo tropism and association with Kaposi's sarcoma*

As described in Section 2.1.5, HHV-8 is consistently found in the vast majority (> 95%) of biopsies from all epidemiological forms of Kaposi's sarcoma, i.e. AIDS-associated Kaposi's sarcoma, classical Mediterranean Kaposi's sarcoma, post-transplant Kaposi's sarcoma and African endemic Kaposi's sarcoma (see Table 15).

HHV-8 has been found by PCR in-situ hybridization in the flat endothelial cells lining ectatic vascular spaces, as well as in spindle cells of Kaposi's sarcoma lesions (Boshoff *et al.*, 1995b). These two cell types represent the bulk of the lesion and this observation is therefore compatible with an important etiopathological role of HHV-8 in the development of Kaposi's sarcoma.

However, primary cultures established from fresh Kaposi's sarcoma biopsies lose HHV-8 after a few passages, and established Kaposi's sarcoma cell cultures, including permanent cell lines (see above) are negative for this virus (Ambroziak *et al.*, 1995; Lebbé *et al.*, 1995). The implications of this observation are unclear.

Therefore, it is possible that the murine angioproliferative lesions induced by Kaposi's sarcoma cell cultures in nude mice are not an adequate model for Kaposi's sarcoma. However, it is also possible that HHV-8 is not required for the development of Kaposi's sarcoma *in vivo* and may only infect and/or replicate preferentially in already established Kaposi's sarcoma endothelial or spindle cells.

In peripheral blood of HIV-infected individuals, HHV-8 is present in B-cells (Ambroziak *et al.*, 1995) and its detection correlates inversely with the number of $CD4^+$ T-cells, suggesting that its replication is under immunological control (Whitby *et al.*, 1995).

Thus, the available evidence suggests that HHV-8 is a strong candidate for the long-sought 'Kaposi's sarcoma agent', but its precise role and epidemiology remain to be established.

4.3 Non-Hodgkin's lymphomas and other lymphoproliferative disorders

As discussed in Sections 2.2 and 2.3.3 and listed in Table 16, the incidence of several types of lymphoproliferative disease is increased in HIV-infected patients.

4.3.1 *Pathological models of lymphomagenesis*

The biological basis and molecular genetics underlying the pathogenesis of AIDS-related non-Hodgkin's lymphomas and other lymphoproliferative disorders (Hodgkin's disease and multicentric Castleman's disease) are not well understood. Several pathological conditions seem to contribute to AIDS-related lymphomagenesis: immunosuppression, dysregulation of cytokine loops, accumulation of genetic lesions within the proliferating clones and infection by viruses (reviewed by Knowles, 1993; Gaidano & Carbone, 1995). These contributory factors may act at different stages of a proposed multistage model of lymphomagenesis (Pelicci *et al.*, 1986; Feichtinger *et al.*, 1992a; Gaidano & Dalla-Favera, 1992; Knowles, 1993; Gaidano *et al.*, 1994a; Herndier *et al.*, 1994b).

The development of AIDS-related non-Hodgkin's lymphoma is often preceded by polyclonal hypergammaglobulinaemia and persistent generalized lymphadenopathy (PGL) (Carbone *et al.*, 1991; Raphael *et al.*, 1991); furthermore, chromosomal abnormalities (Alonso *et al.*, 1987) and oligoclonal immunoglobulin gene rearrangements are detectable in a fraction of these HIV-associated lymphadenopathies (Pelicci *et al.*, 1986; Carbone *et al.*, 1989). Unlike PGL, AIDS-related non-Hodgkin's lymphoma is usually monoclonal and is characterized by a number of molecular alterations of dominantly-acting oncogenes and of tumour-suppressor genes (Ballerini *et al.*, 1993; Gaidano *et al.*, 1993). According to this model of lymphomagenesis, the emergence of oligoclonal B-cell expansions representing a pre-malignant condition is at first driven by several factors including immune dysregulation and viral infections. This phase clinically and pathologically corresponds to PGL. In subsequent phases, the neoplastic transformation of a B-cell clone is due to the accumulation of genetic lesions which eventually transform the clone developing the non-Hodgkin's lymphoma (Figures 10–12). The

Figure 10. Schematic representation of follicle disruption during the course of HIV infection, showing the progression from follicular hyperplasia to follicular involution

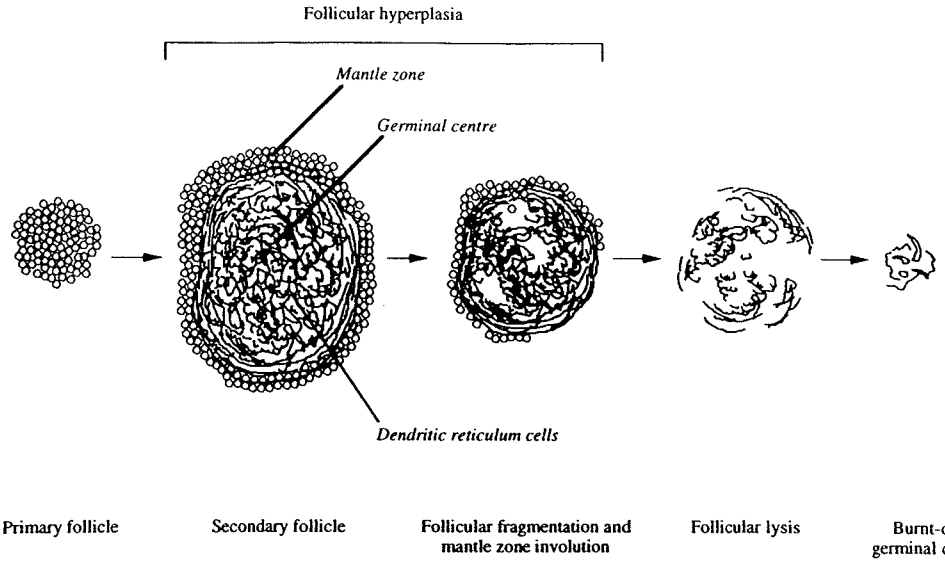
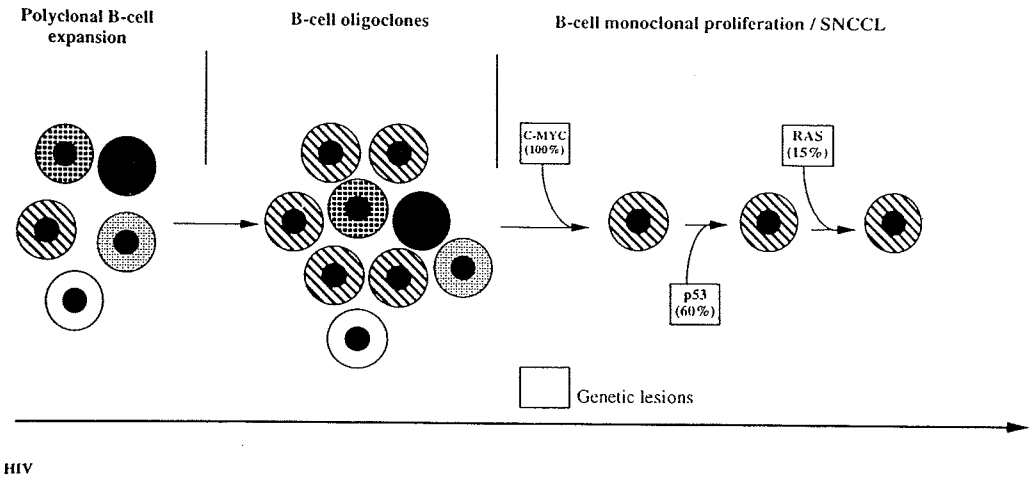


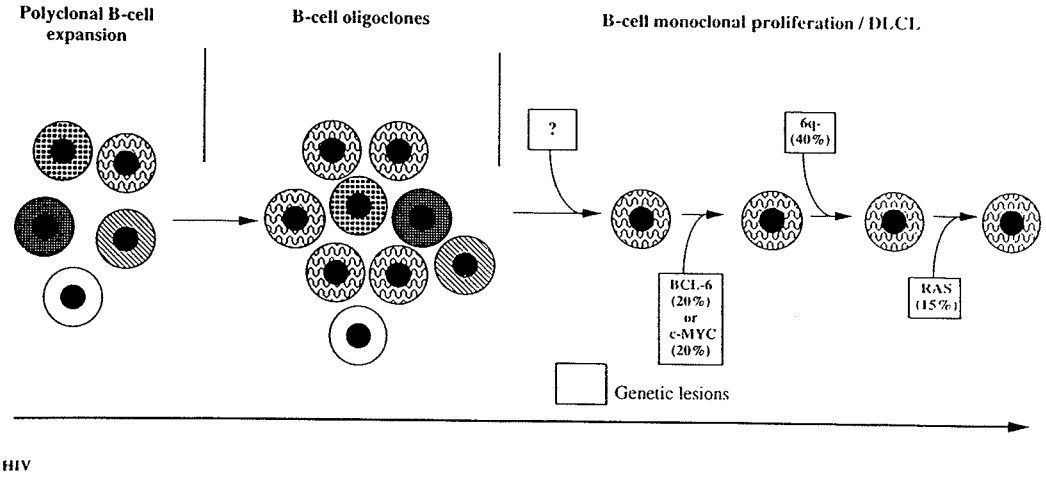
Figure 11. Genetic lesions contributing to pathogenesis of AIDS-related small non-cleaved-cell lymphoma



Adapted from Gaidano *et al.* (1994a)
SNCCL, small non-cleaved cell lymphoma

complex pathophysiological milieu of HIV infection is obviously of importance in the pathogenesis of AIDS-related lymphomas. Morphological, immunopathological, molecular and cytogenetic analyses of the pathological changes in lymphoid tissues during HIV infection have improved the understanding of the mechanisms leading to lymphoma onset and progression.

Figure 12. Genetic lesions contributing to the pathogenesis of AIDS-related diffuse large-cell lymphoma



Adapted from Gaidano *et al.* (1994a)
DLCL, diffuse large-cell lymphoma

As depicted in Figures 11 and 12, three biological stages in the development of B-cell lymphoma can be distinguished: (*a*) polyclonal B-cell hyperplasia; (*b*) oligoclonal expansion and (*c*) genetic changes. Combination of these factors leads to the eventual emergence of monoclonal lymphoma.

(*a*) *HIV infection of lymphoid tissue and polyclonal B-cell hyperplasia*

A critical event in initiating and establishing HIV infection is the localization of HIV in lymphoid organs which become the major reservoirs of HIV and sites of viral replication (Fox *et al.*, 1991). Viral particles and antigen become trapped on the surface of the web-like processes of the follicular dendritic cells which permeate the germinal centres. These cells then expand to form the core of the lymphoid tissue (Biberfeld *et al.*, 1985; Tenner-Rácz *et al.*, 1985; Pantaleo *et al.*, 1993a,b). Persistence of virus in lymphoid organs causes chronic stimulation of the immune system which ultimately leads to degeneration of the follicles (reviewed by Pantaleo & Fauci, 1995). Morphological analyses at different stages of HIV infection have demonstrated that lymphoid tissues undergo progressive destruction and depletion of B-cell areas as the disease advances (Biberfeld *et al.*, 1985, 1987; Ioachim *et al.*, 1990; Fox *et al.*, 1991). The severe immunosuppression at advanced stages of disease is one of the functional consequences of this process (reviewed by Pantaleo & Fauci, 1995). In contrast, lymph-node architecture and immune function appear to be intact in some HIV-infected individuals who remain free of disease for many years (Pantaleo *et al.*, 1995).

(i) *Pathological changes in HIV-infected lymphoid follicles*

The lymph nodes in HIV-infected patients with PGL have been extensively studied both histologically and immunophenotypically (Ioachim *et al.*, 1983; Baroni *et al.*, 1985; Janossy *et al.*, 1985; Wood *et al.*, 1985; Carbone *et al.*, 1986; Wood *et al.*, 1986). The first lymphadenopathic change is follicular hyperplasia (Biberfeld *et al.*, 1985), which is the expansion of the germinal centre by recruitment, proliferation and differentiation of antigen-reactive B-cells (follicular hyperplasia). Morphologically, follicles appear to be increased in size and number and show a marked variation in shape and irregular marginal zones. By immunohistochemical methods, a colocalization of HIV p24 antigen with follicular dendritic cells is clearly visible in the secondary germinal centres (Biberfeld *et al.*, 1985; Baroni *et al.*, 1986). Follicular fragmentation, which may represent an early degenerative change, can be perceived as a disruption of the dendritic reticulum of the germinal centre (Biberfeld *et al.*, 1985). Also, the follicular mantle zones become progressively reduced (Wood *et al.*, 1985). Such follicular changes in lymph nodes have also been detected in mucosal, 'hypertrophic' nasopharyngeal lymphoid tissue (Barzan *et al.*, 1989; Shahab *et al.*, 1994). Nasopharyngeal lymphoid tissue 'hypertrophy', often associated with PGL (Barzan *et al.*, 1990), is apparently linked to the early phase of HIV infection in the same way as follicular hyperplasia is in PGL (Carbone *et al.*, 1995b).

As HIV disease progresses, germinal centres show a reduction in the number of $CD4^+$ T-lymphocytes and an increase in the percentage of $CD8^+$ T-cells (Modlin *et al.*, 1983b; Said *et al.*, 1984; Carbone *et al.*, 1985; Biberfeld *et al.*, 1986), reflecting the decrease in $CD4^+ : CD8^+$ lymphocyte ratio of peripheral blood. The destruction of the follicular dendritic cell network and the collapse of the germinal centres become increasingly evident (the so-called burning-out phenomenon) (Biberfeld *et al.*, 1985). Follicular involution is characterized by hypervascularity, with small follicles resembling those seen in multicentric Castleman's disease. Germinal centres are small and show hyalinization and fibrosis (Figure 10).

These pathological changes, ranging from follicular hyperplasia to follicular involution, usually involve most lymphoid tissue, including tonsils, abdominal lymph nodes and spleen (Burke *et al.*, 1993).

(ii) *Destruction of follicular centres and B-cell hyperplasia*

It has been suggested that abnormal B-cell proliferation takes place when follicular architecture is disrupted by HIV (Armstrong & Horne, 1984; Tenner-Rácz *et al.*, 1985; Feichtinger *et al.*, 1992a). According to one version of this hypothesis, the destruction of follicular dendritic cells interferes with apoptosis and allows the proliferation of B-cell clones expressing low-avidity cell surface immunoglobulin (Herndier *et al.*, 1994b). Another aspect is the dissemination of follicular dendritic cells outside of lymphoid tissue, which could permit the formation of germinal centres in non-lymphoid tissue from which a polyclonal B-cell proliferation and B-cell lymphoma would emerge (Feichtinger *et al.*, 1992a; Herndier *et al.*, 1994b).

(iii) *Chronic antigen stimulation*

Chronic antigen stimulation, pathologically observed as florid B-cell hyperplasia, has been postulated to be a key factor in Burkitt's lymphoma pathogenesis in patients with AIDS (reviewed by Karp & Broder, 1992). Evidence for this is the finding that AIDS-related Burkitt's lymphomas frequently produce antibodies directed against self antigens; furthermore, the hypervariable regions of the immunoglobulin genes utilized by AIDS-related Burkitt's lymphoma carry somatic mutations, which may have been selected by antigen stimulation (Ng *et al.*, 1994; Riboldi *et al.*, 1994). Together, these data suggest that a process of B-cell clonal selection is involved in AIDS lymphomagenesis.

(iv) *Presence of HIV in tumour cells*

Tumours from AIDS-related non-Hodgkin's lymphoma are almost all of B-cell origin. In these tumours, HIV has not been detected in the B-lymphocytes. For example, Morgello (1992) reported a series of 12 primary central nervous system lymphomas from New York, United States. None was positive for HIV *gag* sequences by the sensitive technique of PCR. Similarly, Cornford *et al.* (1991) studied the immunohistochemical localization of HIV in seven cases of central nervous system lymphoma in Los Angeles, CA, United States. While they detected HIV near the mass lesions in five (70%) of the cases, in no instance was HIV detected within the neoplastic lymphoid cells themselves.

Insertional mutagenesis with a direct role of HIV has been proposed to explain some cases of AIDS-related non-Hodgkin's lymphoma. Shiramizu *et al.* (1994) reported four cases that had HIV clonally integrated in the tumour. In one case of T-cell immunophenotype, HIV was detectable in T-cells by anti-p24 immunostaining. The other three cases having a B-, T- or null phenotype contained a large histiocytic reactive component; HIV was localized to these reactive cells. All four cases were reported to have a common integration site of HIV upstream from the c-*fes/fps* proto-oncogene, which suggested an insertional mutagenesis role for HIV in a subset of AIDS-related lymphomas.

In another study, it was also suggested that HIV could play a direct role in B-cell transformation. This was based on the increased proliferation *in vitro* of B-lymphocytes dually infected with HIV and EBV (Laurence & Astrin, 1991). In addition, Astrin *et al.* (1992) reported detection by PCR of, on average, one HIV proviral DNA copy per cell in B-lymphoma tissue, but did not observe monoclonal integration of HIV DNA in B-lymphoma cells. These results therefore fall short of confirming a direct oncogenic effect of HIV in B-cells.

Indeed, consistent failure to detect HIV sequences unequivocally within the tumour clone has suggested that HIV is not directly involved in the development of malignancy (reviewed by Knowles, 1993).

(*b*) *Oligoclonal B-cell proliferation*

Three main groups of cofactors, cytokines, lymphotropic viruses and genetic changes, are thought to be involved during the transition from polyclonal B-cell proliferation to the expansion of oligoclonal B-cell populations.

(i) *Immunosuppression*

As for some other cancers in AIDS, immunosuppression also predisposes to the frequent development of B-cell lymphoma (reviewed by Gaidano & Dalla Favera, 1992; Karp & Broder, 1992).

The relation between immunosuppression and the development of lymphoma is recognized in several clinical conditions other than AIDS, including congenital and iatrogenic immunodeficiences (Frizzera, 1994) (see Sections 2.2.1 and 4.1.3). The relative risk for AIDS-related non-Hodgkin's lymphoma increases with progressive immune dysfunction (Section 2.2.1) (Pluda *et al.*, 1993). Immunosurveillance is known to play an important role in controlling the replication of EBV-infected B-lymphocytes in humans (Rickinson *et al.*, 1992). The specific importance of cytotoxic T-lymphocytes (CTLs) in the control of virus-associated lymphoproliferative disease in immunosuppression has been demonstrated in animal studies by Boyle *et al.* (1993). They showed that EBV-specific CTLs adaptively transferred into SCID mice engrafted with EBV-transformed and immortalized B-lymphoblastoid cell lines delayed or prevented the development of B-cell lymphomas. In another study, five patients who developed EBV-associated lymphoproliferative disease following bone marrow transplantation were given infusions of leukocytes from the original donors. The proliferating cells were of donor cell origin and contained EBV DNA which was clonally integrated in two out of the three cases adequate for study. Since the lymphoproliferation derived from donor cells, the leukocytes included EBV-sensitized CTLs. Complete responses, pathological or clinical, were sustained in the three surviving patients (Papadopoulos *et al.*, 1994). EBV-specific CTLs are now generated in some clinical centres for the prevention and treatment of EBV-associated lymphoproliferative disease or treatment of organ transplant recipients (Smith *et al.*, 1995). The impaired immunosurveillance in AIDS patients may give rise to the oligoclonal B-cell expansion seen in PGL (Birx *et al.*, 1986). Consistently, one third of hyperplastic lymph nodes from HIV-infected individuals with PGL contain EBV-positive clones (Shibata *et al.*, 1991). The presence of EBV-containing B-cell clones in PGL correlates with the simultaneous occurrence or subsequent development of EBV-containing non-Hodgkin's lymphoma (Shibata *et al.*, 1991). However, Dolcetti *et al.* (1995) only rarely observed monoclonal EBV episomes in PGL samples with a high content of EBV-infected cells.

(ii) *Cytokines*

Dysregulation of the normal 'steady-state' cytokine network is a key feature of HIV infection (Fauci *et al.*, 1991). However, data regarding the role of cytokines in AIDS-related lymphomagenesis are restricted to IL-6 and IL-10.

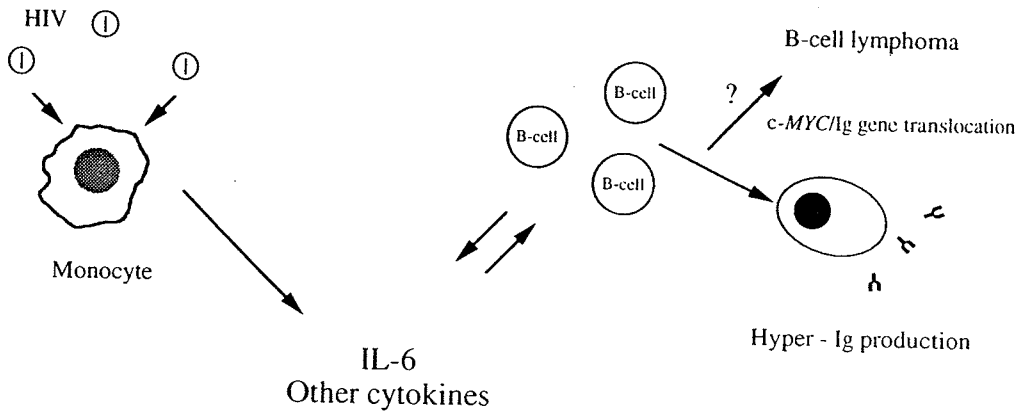
IL-6

The role of IL-6 is schematically depicted in Figure 13. IL-6 may be particularly important to both pre-malignant polyclonal B-cell expansion and malignant transformation.

Monocytes appear to be responsible for the major portion of IL-6 produced by PBMCs isolated from HIV-infected individuals (Birx *et al.*, 1990). The production of

IL-6 by HIV-infected monocytes promotes the proliferation of B-cells activated by, for example, EBV, thereby driving immunoglobulin synthesis and causing the non-specific hyperimmunoglobulinaemia commonly seen in early HIV infection (Birx *et al.*, 1986, 1990). Therefore, IL-6 excess in HIV infection seems to contribute to B-cell hyperstimulation and to hypergammaglobulinaemia (reviewed by Martínez-Maza, 1992) (Figure 13). Moreover, AIDS-related large-cell lymphomas containing a high proportion of immunoblasts express high levels of IL-6 (Emilie *et al.*, 1992). This finding is consistent with the role of IL-6 in the terminal differentiation of B cells. Further evidence linking IL-6 to AIDS-related lymphomagenesis is that HIV-infected patients with elevated serum levels of IL-6 are at high risk for later developing large-cell lymphomas (Pluda *et al.*, 1993). It has also been suggested that, once the lymphoma is well established, continuous tumour growth may be sustained by IL-6 through paracrine loops (Emilie *et al.*, 1992). Thus, IL-6 could contribute to lymphomagenesis either by acting as a chronic stimulus to B cells in HIV-infected people and/or, more directly, as an autocrine or paracrine growth factor for lymphoma cells (Martínez-Maza, 1992).

Figure 13. Potential role of IL-6 in AIDS-related lymphomagenesis



Contact between monocytes and HIV can cause IL-6 production. This increased IL-6 production could then induce B-cell hyperstimulation (hypergammaglobulinaemia) and, possibly, B-cell lymphoma.
Adapted from Martínez-Maza (1992)

An environment of dysregulated cytokines may also play a role in the pathogenesis of AIDS-related body cavity-based lymphomas that usually contain HHV-8 gene sequences. A recent study has demonstrated that IL-6 and IL-10 levels in lymphomatous effusions are much higher than those in normal plasma (Ng *et al.*, 1995). IL-6 protein has also been found in multicentric Castleman's disease (Yoshizaki *et al.*, 1989), another HHV-8-associated lymphoproliferative disorder (Soulier *et al.*, 1995). However, the functional relationship between IL-6 and HHV-8 needs to be clarified further (Levy, 1995).

In conclusion, it is clear that IL-6 is involved in B-cell lymphocyte expansion and could be involved at any stage during the development of B-cell lymphomas (Figure 13).

IL-10

IL-10, a potent B-cell stimulator, is a pleotropic cytokine sharing significant homology with the EBV protein BCRF1. Although the precise role of IL-10 in the development of AIDS-related lymphomagenesis is still unclear, a possible involvement is suggested by the finding that high levels of IL-10 are constitutively expressed by EBV-positive B-cell lines derived from patients with AIDS-related small non-cleaved-cell lymphoma (Benjamin *et al.*, 1992). Furthermore, an autocrine growth mechanism involving IL-10 can occur in AIDS-related lymphoma cells (Masood *et al.*, 1995).

(*c*) *Genetic abnormalities*

Various genetic abnormalities have been found in AIDS-related non-Hodgkin's lymphoma (Ballerini *et al.*, 1993; Gaidano *et al.*, 1993) (see Table 32 and Figures 11 and 12).

Table 32. Frequency of genetic lesions in AIDS-related non-Hodgkin's lymphomas

Histology	c-*myc*	*p53*	*BCL*-6	6q deletions	*ras*	EBV	HHV-8
Small non-cleaved-cell lymphomas (Ballerini *et al.*, 1993; Hamilton-Dutoit *et al.*, 1993a; Gaidano *et al.*, 1994b; Cesarman *et al.*, 1995; Carbone *et al.*, 1996b; Pastore *et al.*, 1996)[a]	100%	60%	Neg.	Neg.	15%	30%	Neg.
Diffuse large B-cell lymphomas (Ballerini *et al.*, 1993; Hamilton-Dutoit *et al.*, 1993a; Gaidano *et al.*, 1994b; Cesarman *et al.*, 1995; Pastore *et al.*, 1996)	20%	Neg.	20%	40%	15%	80%	Neg.
Anaplastic large-cell (CD30/Ki-1$^+$) lymphomas (Carbone *et al.*, 1993b; Chadburn *et al.*, 1993; Cesarman *et al.*, 1995; Carbone *et al.*, 1996b; Pastore *et al.*, 1996)	Neg.	Neg.	ND	Neg.	ND	90%	Neg.
Body cavity-based lymphomas (Cesarman *et al.*, 1995; Carbone *et al.*, 1996a)	Neg.	Neg.	ND	ND	Neg.	> 50%	> 70%

ND, not done

[a] Chromosome 1q abnormalities have been detected in AIDS-related small non-cleaved-cell lymphomas (Bernheim & Berger, 1988; Polito *et al.*, 1995)

(i) *c*-myc

Several reports have pointed to an association of AIDS-related non-Hodgkin's lymphoma with chromosomal translocations involving the c-*myc* oncogene. Activation

of c-*myc* has been detected in 100% of AIDS-related small non-cleaved-cell lymphomas, including Burkitt's lymphoma (Figures 11 and 14). In diffuse large-cell lymphomas including large non-cleaved-cell lymphomas and large-cell immunoblastic plasmacytoid lymphomas, activation is restricted to a minority (approximately 20%) of tumours (Ballerini *et al.*, 1993; Delecluse *et al.*, 1993; Bhathia *et al.*, 1994). Tumours with an intermediate morphology between small non-cleaved-cell and large-cell immunoblastic lymphomas have been shown to harbour a c-*myc* rearrangement. This finding is consistent with the notion that such a tumour may represent a small non-cleaved-cell lymphoma that has adopted an immunoblastic morphotype in the context of AIDS (Delecluse *et al.*, 1993). In contrast, no AIDS-related anaplastic large-cell lymphoma or body cavity-based lymphoma has shown c-*myc* alterations (Chadburn *et al.*, 1993; Cesarman *et al.*, 1995).

As in sporadic Burkitt's lymphoma, c-*myc* activation in AIDS-related non-Hodgkin's lymphoma occurs through gene rearrangements following chromosomal translocations between 8q24, the site of the c-*myc* proto-oncogene, and an immunoglobulin chromosomal locus, most commonly the immunoglobulin heavy-chain genes at 14q32 (Chaganti *et al.*, 1983). B-lymphocyte clones harbouring similar translocations can persist and be detected in peripheral blood of lymphoma-free HIV-positive homosexual men but are rare in HIV-negative controls (Müller *et al.*, 1995).

(ii) BCL-6

Chromosomal translocations in AIDS-related non-Hodgkin's lymphoma also involve *BCL*-6, a proto-oncogene affecting B-cell maturation, that maps to 3q27 (Ye *et al.*, 1993). Gross rearrangements of *BCL*-6 are mostly associated with AIDS-related diffuse large-cell lymphomas (20%) (Figures 12 and 15), and are consistently absent in AIDS-related small non-cleaved-cell lymphomas. This is similar to the chromosomal aberrations seen in the same histological subtypes of non-HIV-related non-Hodgkin's lymphoma. In diffuse large-cell lymphoma, gross rearrangements of *BCL*-6 and of c-*myc* appear to be mutually exclusive genetic lesions (Gaidano *et al.*, 1994b).

(iii) ras

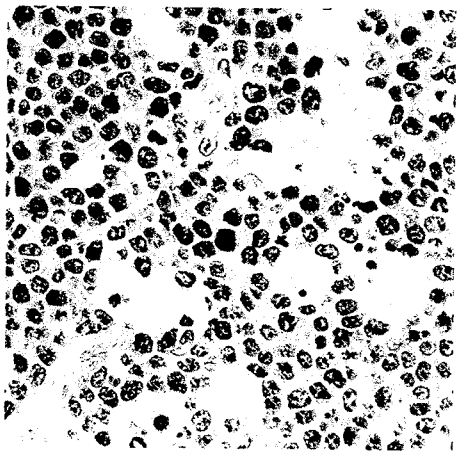
Other dominantly acting oncogenes commonly involved in the pathogenesis of lymphomas in immunocompetent hosts (e.g., *BCL*-1, *BCL*-2) do not seem to play a role in AIDS-related lymphomagenesis (reviewed by Gaidano & Dalla-Favera, 1992). On the other hand, mutations of K-*ras* or N-*ras* genes, which have not been detected in B-cell non-Hodgkin's lymphoma of immunocompetent hosts, were present in 4/27 (15%) of AIDS-related non-Hodgkin's lymphoma (Ballerini *et al.*, 1993).

(iv) p53

A role of tumour-suppressor gene inactivation in AIDS-related lymphomagenesis is supported by a number of observations. Mutations and/or losses of *p53* have been found in 60% of AIDS-related small non-cleaved-cell lymphomas (Ballerini *et al.*, 1993; Gaidano *et al.*, 1993), but not in the other types of AIDS-related non-Hodgkin's lymphoma (Gaidano *et al.*, 1991; Ballerini *et al.*, 1993; De Re *et al.*, 1994). In the small non-cleaved-cell lymphomas series examined (Ballerini *et al.*, 1993), *p53* mutations were

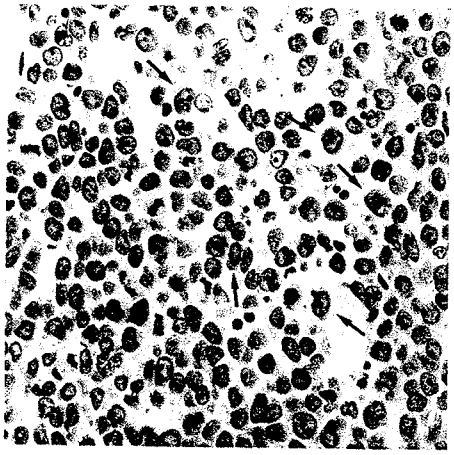
Figure 14

Small non-cleaved-cell lymphoma



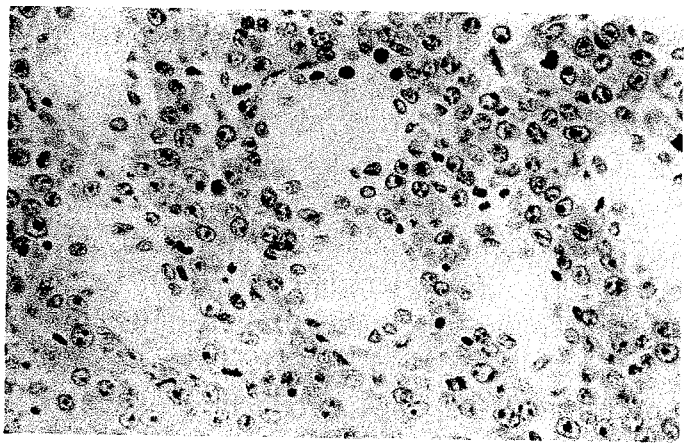
The tumour is composed of small to medium-sized monomorphic cohesive cells interspersed with large phagocytosine histiocytes (starry sky pattern). Haematoxylin–eosin, × 400

Small non-cleaved-cell lymphoma with plasma cell differentiation



Tumour cells have round or irregular, frequently eccentric, nuclei containing randomly located nucleoli. Larger basophilic cells with large nucleoli are recognizable (arrows). Haematoxylin–eosin, × 400

Figure 15. Diffuse large-cell lymphoma of the immunoblastic type with plasmacytic features



Gastric involvement by diffuse large-cell lymphoma of the immunoblastic type with plasmacytic features. Most tumour cells have large, solitary nucleoli. In this field mucosal glandular epithelium is surrounded, but not destroyed, by tumour growth. Haematoxylin–eosin, × 400.

seen only in tumours carrying a rearranged c-*myc* gene. p53 protein overexpression was observed in 3/3 lymphomas with a morphology intermediate between small non-cleaved-cell and large-cell immunoblastic lymphomas. It is unknown whether this overexpression was due to *p53* mutations (Carbone *et al.*, 1995b).

Little is known about the frequency of *p53* aberrations in anaplastic large-cell lymphomas. In contrast to small non-cleaved cell lymphoma, AIDS-related anaplastic large-cell lymphoma has been reported not to contain *p53* mutations, but accumulation of wild-type p53 protein has been observed by immunohistochemistry (Inghirami *et al.*, 1994; Carbone *et al.*, 1996b), as reported previously for this type of lymphoma in immunocompetent hosts (Cesarman *et al.*, 1993).

(v) *6q deletions*

Deletions of the long arm of chromosome 6 at band q27 occur in non-Hodgkin's lymphoma (both AIDS-related and -unrelated) and represent the putative site of a distinct tumour-suppressor gene. 6q deletions among AIDS-related non-Hodgkin's lymphoma were restricted to diffuse large-cell lymphomas (5/13 cases) (Pastore *et al.*, 1996), whereas, among non-Hodgkin's lymphoma in immunocompetent hosts, 6q deletions occur throughout the entire histological spectrum, including both diffuse large-cell and small non-cleaved-cell lymphomas (Gaidano *et al.*, 1992).

(vi) *Chromosome 1q abnormalities*

In AIDS-related small non-cleaved-cell lymphomas, structural changes of chromosome 1 have been found (Bernheim & Berger, 1988). Cell lines derived from such tumours have also been found to contain chromosome 1q abnormalities (Polito *et al.*, 1995). These chromosomal changes are very similar to those previously detected in AIDS-unrelated small non-cleaved-cell lymphomas or cell lines (Gurtsevitch *et al.*, 1988; Kornblau *et al.*, 1991). Owing to its very frequent involvement, chromosome 1q 21-25 is a site that should be examined in greater detail for genetic alterations that may play a pathogenetic role in small non-cleaved-cell lymphomas (Polito *et al.*, 1995).

4.3.2 *Lymphotropic viruses*

(*a*) *EBV*

EBV appears to play an important role in the development of some AIDS-related non-Hodgkin's lymphoma (Knowles, 1993; Herndier *et al.*, 1994b; Rabkin, 1994). The best evidence so far for its pathogenetic role is the ability of EBV-infected B cells to cause EBV-positive B-cell lymphomas in SCID mice (Mosier *et al.*, 1989; Rowe *et al.*, 1991). Other studies have demonstrated that the introduction of activated c-*myc* genes into EBV-transformed lymphoblasts confers tumorigenicity in nude mice (Lombardi *et al.*, 1987).

HIV-infected individuals possess abnormally high numbers of circulating EBV-infected B cells (Birx *et al.*, 1986). Moreover, EBV infection precedes the expansion of the tumour clone (Neri *et al.*, 1991), and a large fraction (see below) of AIDS-related non-Hodgkin's lymphoma cells contain EBV sequences and express at least some EBV

latent proteins known to have transforming properties (Hamilton-Dutoit *et al.*, 1989; Ballerini *et al.*, 1993; Hamilton-Dutoit *et al.*, 1993b).

It is likely that HIV-related immunosuppression permits the development of EBV-infected and immortalized B-cell clones. Such clones are susceptible to further genetic alterations resulting in the development of an EBV-containing monoclonal lymphoproliferation (Pelicci *et al.*, 1986).

The frequency of EBV infection in AIDS-related non-Hodgkin's lymphoma has been a matter of controversy (reviewed by Gaidano *et al.*, 1994a; Shibata, 1994). Discrepancies may depend on the different methods used for viral detection (Southern blot, PCR or in-situ hybridization) and on the different histological types or sites of disease investigated.

In contrast to systemic non-Hodgkin's lymphoma, AIDS-associated primary lymphomas of the central nervous system were positive for EBV in most studies (MacMahon *et al.*, 1991; Hamilton-Dutoit *et al.*, 1993a; Camilleri-Broët *et al.*, 1995; Cinque *et al.*, 1993) (see Table 20). However, Gunthel *et al.* (1994) reported a few primary lymphomas of the central nervous system that were negative for EBV by a sensitive PCR assay. Almost all lymphomas primarily involving body cavities contain clonal EBV genome (Knowles *et al.*, 1989; Cesarman *et al.*, 1995).

Most molecular studies have indicated that the presence of EBV within systemic AIDS-related non-Hodgkin's lymphoma varies according to the histopathological type (Table 21). EBV infection is found in the majority of diffuse large-cell lymphomas, particularly in the large-cell immunoblastic lymphoma subtype (80%), but in a much smaller fraction (30–50%) of small non-cleaved-cell lymphomas (Hamilton-Dutoit *et al.*, 1991; Ballerini *et al.*, 1993). A high frequency of EBV association has been shown in anaplastic large-cell lymphoma (80–90%) and Hodgkin's disease (90–100%) tissues from AIDS patients (Carbone *et al.*, 1993a; Hamilton-Dutoit *et al.*, 1993a; Tirelli *et al.*, 1995b). The EBV genomes in such cases have been reported to be episomal and clonal (Boiocchi *et al.*, 1993a), even when detected in multiple, independent lesions (Boiocchi *et al.*, 1993b).

There are two EBV subtypes which differ in the genomic region encoding the EBV nuclear antigen-2 (EBNA-2) (Adldinger *et al.*, 1985). Type 1 EBV is a more potent lymphocyte transformer than type 2 (Rickinson *et al.*, 1987). While type 2 virus rarely occurs in immunocompetent hosts in developed countries, it is found in a much higher proportion of subjects with HIV-related immunosuppression. The elevated frequency of type 2 virus in AIDS-related lymphoproliferative diseases appears to mirror the excess seen in HIV-infected subjects without such disease (Boyle *et al.*, 1991, 1993; De Re *et al.*, 1993).

A role of EBV in the pathogenesis of AIDS-related non-Hodgkin's lymphoma is further supported by data showing that the EBV-transforming proteins, EBV-encoded latent membrane protein-1 (LMP-1) and/or EBNA-2 may be expressed in EBV-positive cases.

Expression of LMP-1 has been detected in AIDS-related lymphomas of various localizations and histological types. In primary AIDS-related immunoblastic lymphomas of

the central nervous system, 10/11 (90%) of tumours expressed LMP-1 and 21/57 (54%) expressed EBNA-2, as assessed by immunohistochemistry. Expression of both *BCL-2* and LMP-1 in EBV-positive AIDS-related primary brain lymphomas *in vivo* has been described (Camilleri-Broët *et al.*, 1995). This is in agreement with in-vitro findings showing that *BCL-2* can be transactivated by LMP-1 in small non-cleaved-cell lymphoma cell lines. Also, *BCL-2* expression induced by LMP-1 may protect tumour B cells from apoptosis and lead to a higher proliferative rate (Henderson *et al.*, 1991; Finke *et al.*, 1992). Body cavity-based lymphoma cells exhibiting pleomorphic and anaplastic morphology are also associated with LMP-1 expression (Carbone *et al.*, 1996a,c).

Regarding AIDS-related systemic lymphomas, some investigators have reported that LMP-1 expression is restricted to anaplastic large-cell lymphomas (Carbone *et al.*, 1993a, 1994) and Hodgkin's disease (Audouin *et al.*, 1992; Carbone *et al.*, 1993a; Siebert *et al.*, 1995), while AIDS-related large-cell immunoblastic lymphomas show heterogeneity in both EBV presence and latency patterns (Carbone *et al.*, 1993a; Hamilton-Dutoit *et al.*, 1993b). In Hodgkin's disease, EBV adopts a latency type 2 pattern (LMP-1$^+$, EBNA-2$^-$) (Boiocchi *et al.*, 1993a), while AIDS-associated anaplastic large-cell lymphomas appear to be heterogeneous and both the type 2 patterns and, less frequently, a type 3 pattern (LMP-1$^+$, EBNA-2$^+$ phenotype) have been described (Carbone *et al.*, 1996b).

In contrast, EBV-positive AIDS-related small non-cleaved-cell lymphomas usually show the restricted latency pattern of EBV gene expression (latency type 1 pattern; EBNA-1$^+$, EBNA-2$^-$, LMP-1$^-$) also found in endemic Burkitt's lymphoma (Carbone *et al.*, 1993a; Hamilton-Dutoit *et al.*, 1993a). However, in some EBV-positive cases of small non-cleaved-cell lymphoma, a limited number of tumour cells express LMP-1 but not EBNA-2 (Hamilton-Dutoit *et al.*, 1993b; Carbone *et al.*, 1996b). Furthermore, both EBV latency type 2 pattern and a new latency pattern (EBNA-2$^+$, LMP-1$^-$) have been found in endemic, sporadic and AIDS-related small non-cleaved-cell lymphomas (Niedobitek *et al.*, 1995; Carbone *et al.*, 1996c). Altogether, these data document heterogeneous expression of EBV latent proteins throughout the entire spectrum of small non-cleaved-cell lymphomas.

LMP-1 expression has not been found in cases of EBV-associated plasmacytomas (Voelkerding *et al.*, 1989; Carbone *et al.*, 1993a).

In summary, EBV is more frequently present in large-cell AIDS-related lymphomas, including body cavity-based lymphomas, large-cell immunoblastic lymphomas, either systemic or arising in the brain, and anaplastic large-cell lymphomas. The two subtypes of EBV (types 1 and 2) are almost equally represented, and three types of EBV latency pattern (latency 1 — EBNA-1$^+$, EBNA-2$^-$, LMP-1$^-$; latency 2 — EBNA-1$^+$, EBNA-2$^-$, LMP-1$^+$; latency 3 — EBNA-1$^+$, EBNA-2$^+$, LMP-1$^+$) have been detected. Therefore, a large fraction of AIDS-related diffuse large-cell lymphomas can be considered as EBV-driven lymphoproliferations arising in the absence of effective cell-mediated immunity against EBV. Since EBV-transforming antigens are expressed by EBV-positive AIDS-related diffuse large-cell lymphomas, it is plausible that EBV is indeed a driving force for tumour growth and expansion. Moreover, while Hodgkin's disease may not be more

common in HIV-infected persons, it is more frequently associated with EBV infection in such individuals.

The grouping of the different pathological subtypes of AIDS-related lymphomas based on EBV association and EBV latent gene expression is shown in Table 33 (see also Figure 16).

Table 33. Grouping of pathological types of AIDS-related lymphomas based on EBV latent gene expression and genetic abnormalities

'Blastic'[a] cell lymphomas not associated with expression of Epstein–Barr virus-encoded latent membrane protein-1

- Large non-cleaved cell
- Small non-cleaved cell (always associated with c-*myc* rearrangements and frequently with *p53* inactivation)
- Extramedullary (plasmacytoma)[b]
- Blastic cells with 'intermediate' features

'Blastic'[a] cell lymphomas that may be associated with expression of Epstein–Barr virus-encoded latent membrane protein-1 expression

- Immunoblastic (either systemic or arising in the brain as a primary site)
- Occasional cases of small non-cleaved cell

'Anaplastic'[b] cell lymphomas associated with monoclonal Epstein–Barr virus infection and latent membrane protein-1 expression

- Anaplastic large-cell (CD30/Ki-1$^+$) lymphomas
- Body cavity-based lymphomas (associated with HHV-8 infection)[d]
- Hodgkin's lymphoma (mixed cellularity and lymphocyte depletion)[b]

Updated and adapted from Carbone *et al.* (1993b)

[a]The term 'blastic' is used in analogy with the suffix 'blastic' used in the Kiel classification (Stansfeld *et al.*, 1988).

[b]Whether extramedullary plasmacytomas and Hodgkin's lymphomas should be included among HIV-related lymphomas is still debated.

[c]The term 'anaplastic' is used in analogy with the term used in the definition of CD30-positive anaplastic large-cell lymphomas; it indicates blastic large cells which display marked pleomorphism, with giant cells possessing bizarre and irregular nuclei and large nucleoli (Harris *et al.*, 1994).

[d]The morphology of body cavity-based lymphoma cells includes both immunoblastic and anaplastic features (Ansari *et al.*, 1996)

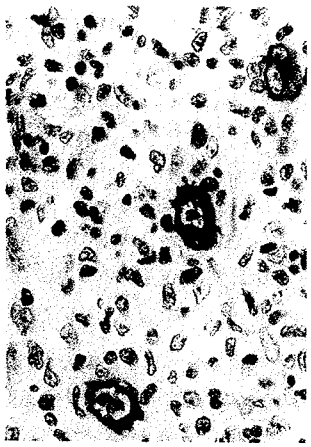
The frequent association between EBV infection and some lymphomas in HIV-positive persons, including those arising primarily in the brain and body cavities, as well as anaplastic large-cell lymphoma and Hodgkin's disease types, suggests that EBV is an important cofactor in their pathogenesis. Thus, the presence of EBV in these lymphoma cells appears important for their neoplastic transformation as well as for the expression of certain morphological and immunophenotypic features in the context of HIV infection (Cesarman *et al.*, 1995; Gaidano & Carbone, 1995). This conclusion is consistent with the observation discussed in Section 3.2.2, indicating that B-cell lymphoma in SIV-infected macaques is frequently associated with an EBV-related virus.

Figure 16

EBV-encoded latent membrane protein-1 (LMP-1) expression in AIDS-related CD30$^+$ anaplastic large cell lymphoma

Several large tumour cells show a strong cytoplasmic staining. Bouin-fixed paraffin-embedded tissue section, APAAP method, haematoxylin counterstain, × 400

EBV-encoded latent membrane protein-1 (LMP-1) expression in Hodgkin's disease



Reed–Sternberg cells of Hodgkin's disease, mixed cellularity subtype, show strong cytoplasmic staining for EBV-encoded LMP-1. Bouin-fixed paraffin-embedded tissue section, APAAP method, haematoxylin counterstain, × 400

(*b*) *HHV-6*

Human herpesvirus-6 (HHV-6) is a member of the herpesviridae family, and was originally isolated from peripheral blood mononuclear cells of patients with lymphoproliferative disorders or AIDS (Salahuddin *et al.*, 1986). HHV-6, like the human retroviruses HIV, HTLV-I and HTLV-II, predominantly infects T lymphocytes but can also infect other cell types including fibroblasts, epithelial cells, natural killer cells, megakaryocytes, neural cells and, occasionally, B lymphocytes.

Like other herpesviruses, HHV-6 is responsible for a latent, lifelong infection of the host and can reactivate during immunosuppression (Carrigan *et al.*, 1991; Knox & Carrigan, 1994).

The role of this virus in the pathogenesis of AIDS-related non-Hodgkin's lymphoma is still obscure. It has been hypothesized that HHV-6 may contribute to the development of lymphoproliferative disorders by stimulating polyclonal B-cell activation as a consequence of persistent active viral infection (Krueger *et al.*, 1989). A combined molecular and immunohistochemical study has shown that HHV-6 DNA sequences are significantly more prevalent in persistent generalized lymphadenopathy biopsies than in HIV-unrelated reactive lymphadenopathies. The presence of HHV-6 sequences closely correlates with follicular hyperplasia, while follicular involution is HHV-6-negative. Therefore, persistent generalized lymphadenopathy lymph nodes with B-cell hyperplasia

constitute one of the sites where biologically relevant interactions between HHV-6 and HIV may occur (Dolcetti *et al.*, 1996).

However, the prevalence of HHV-6 DNA in Hodgkin's disease and B-cell non-Hodgkin's lymphoma from HIV-infected patients is remarkably low (Carbone *et al.*, 1996b) and similar to that observed in lymphoproliferative disorders from HIV-seronegative patients (Di Luca *et al.*, 1994; Dolcetti *et al.*, 1996). These results suggest that HHV-6 may have no direct role in the pathogenesis of AIDS-related non-Hodgkin's lymphoma and Hodgkin's disease.

(*c*) *HHV-8*

HHV-8 (see Section 4.2.4) has been associated with several lymphoproliferative disorders. It has been found in the majority of body cavity-based lymphomas arising in patients with or without HIV infection (Cesarman *et al.*, 1995; Karcher & Alkan, 1995; Nador *et al.*, 1995; Pastore *et al.*, 1995) as well as in all (14/14) HIV-associated and a proportion (21/75) of HIV-unrelated multicentric Castleman's disease tissues (Soulier *et al.*, 1995). Both in fresh body cavity-based lymphoma samples and in cell lines derived from such tumours, HHV-8 is present in multiple episomal copies. Body cavity-based lymphomas are frequently co-infected with EBV (Cesarman *et al.*, 1995), but a few cases which contain only HHV-8 have been reported (Renne *et al.*, 1996) and a few others do not contain HHV-8 (Carbone *et al.*, 1996b; Hermine *et al.*, 1996).

Cell lines latently infected with HHV-8 and several cases also with EBV have been established from body cavity-based lymphoma effusions (Cesarman *et al.*, 1995; Gaidano *et al.*, 1996). HHV-8 is also present in peripheral blood B cells in some HIV-infected individuals with neither lymphoma nor Kaposi's sarcoma (Whitby *et al.*, 1995). In addition, HHV-8 has been detected in PMBCs and lymphoid tissue of less than 10% of HIV-uninfected individuals (Bigoni *et al.*, 1996). The issue of how common HHV-8 is in the general population is discussed in Section 2.1.5.

Whether HHV-8 , like its close relative EBV, is oncogenic in its own right is not yet clear. Mechanisms of pathogenesis that might operate in HHV-8-positive lymphomas include cooperation with EBV, and participation of an HHV-8-encoded cyclin homologue or HHV-8-induced lymphokines (Levy, 1995; Hermine *et al.*, 1996).

4.3.3 *Conclusion*

The putative role of cofactors differs substantially according to the pathological type and site of disease; moreover, several independent pathways in AIDS-related lymphomagenesis can be identified.

The first pathway of pathogenesis is associated with small non-cleaved-cell lymphomas (Figure 11). More than in other AIDS-related non-Hodgkin's lymphomas, antigen stimulation appears to play an important role in this form of non-Hodgkin's lymphoma (Riboldi *et al.*, 1994). At the molecular level, genetic changes appear to be fairly homogeneous (Table 32). They are characterized by rearrangement of c-*myc* (100%), mutation of *p53* (60%) and the presence of EBV infection (30%) (Ballerini

et al., 1993; Gaidano *et al.*, 1993); however, expression of EBV transforming protein is usually absent (Carbone *et al.*, 1993a; Hamilton-Dutoit *et al.*, 1993b).

A second pathway of pathogenesis is associated with diffuse large-cell lymphomas (Figure 12 and Table 32). Because of the very high frequency of EBV infection (60–100%) (Hamilton-Dutoit *et al.*, 1991; MacMahon *et al.*, 1991; Ballerini *et al.*, 1993), AIDS-related diffuse large-cell lymphomas, including those arising primarily in the brain, can be considered as EBV-driven lymphoproliferations developing in the context of a disrupted immunosurveillance against EBV (Birx *et al.*, 1986). Viral transforming proteins EBNA-2 and LMP-1 may be expressed by EBV-positive diffuse large-cell lymphomas (Carbone *et al.*, 1993a; Hamilton-Dutoit *et al.*, 1993b). The vast majority (80–90%) of AIDS-related anaplastic large-cell lymphomas are also associated with EBV infection (Carbone *et al.*, 1993b) (Table 33) and EBV-infected tumour cells consistently express LMP-1 (Carbone *et al.*, 1994) (Figure 16).

A third pathway may apply in the pathogenesis of body cavity-based lymphomas. This pathway includes EBV infection and consistent presence of HHV-8, at least in most cases, but not other known genetic lesions (Cesarman *et al.*, 1995) (Table 32).

Finally, Hodgkin's disease in HIV-infected persons appears to be an EBV-related lymphoma expressing LMP-1 (Audouin *et al.*, 1992; Carbone *et al.*, 1994; Siebert *et al.*, 1995), whereas multicentric Castleman's disease seems to be an HHV-8-related disorder in the HIV setting (Soulier *et al.*, 1995).

In summary, understanding of the mechanisms of lymphomagenesis is hampered by the heterogeneity of non-Hodgkin's lymphoma and the substantial number of cofactors examined. These have been studied independently, generally on relatively small numbers of tumours. Seldom have different mechanisms of lymphomagenesis been examined in the same study.

4.4 Cofactors in anal and cervical carcinomas and other cancers

As discussed in Section 2.3, preneoplastic anogenital lesions and HPV-related changes (koilocytosis) are associated with HIV infection, whereas no such association has been convincingly demonstrated for invasive cancer. Dysregulation of the expression of early proteins E6 and E7 of high-risk HPV types is strongly suggested by in-vitro studies to be an important factor in malignant progression, as well as by data from human tumours (see IARC, 1995).

Little is known about the pathogenetic mechanisms involved in anogenital oncogenesis associated with other viral and chemical agents; indirect and/or direct modulation of HPV expression, however, seems to be the most relevant pathway. There are two possible, not mutually exclusive, ways in which HIV may contribute to HPV-related carcinogenesis: the major indirect mechanism is immunosuppression; possible direct mechanisms include transactivation of HPV oncogenic early-gene expression and abnormal expression of cellular genes.

4.4.1 *The role of HPV in the molecular pathogenesis of anogenital cancers in immunocompetent patients*

HPVs have been recognized as sexually transmitted etiological agents for human lower genital tract malignancies (zur Hausen, 1989; IARC, 1995). Over 70 types of HPV have been identified, of which only a small subset (HPV-16, -18, -31, -33, -35, -45 and, more recently, -51 and -52) have been associated with anogenital cancers. Many more subtypes are associated with benign, epithelial neoplasms. During the life cycle of HPV, most of the viral DNA is maintained episomally in the nucleus of the infected cells. Integration of viral DNA sequences is frequently associated with malignant progression (Schwarz *et al.*, 1985; Jeon *et al.*, 1995), being detected more frequently in carcinomas than in cervical intraepithelial neoplasia (CIN) (Cullen *et al.*, 1991). In CIN, the mainly episomal HPV actively replicates (productive infection), whereas in cervical epithelial cancers the HPV DNA is prevalently integrated (latent infection). This transition results in changes at the level of viral DNA as well as of RNA and protein.

(*a*) *Status and level of HPV DNA in the natural history of infection*

In CIN lesions, the level of predominantly episomal, infecting viral genome detected varies according to the techniques used. PCR, with a detection limit of 10 copies per sample, detects HPV genomes in 72–91% of 'low-grade lesions' and in 90–100% of 'high-grade lesions' (van den Brule *et al.*, 1991; Bergeron *et al.*, 1992; Lungu *et al.*, 1992); procedures with a lower sensitivity (3×10^5 viral genomes per sample or 0.1 viral copy per cell when testing 1.5×10^5 cells = 1 μg genomic DNA), such as Southern blot, dot blot and ViraPap™, detect HPV genomes in only 36–55% of 'low-grade lesions' and in 43–81% of 'high-grade lesions' (Fuchs *et al.*, 1988; Lim-Tan *et al.*, 1988; McNicol *et al.*, 1989).

In invasive cancer, HPV DNA is present at > 1 viral copy per cell, because of the predominantly integrated high-risk HPVs in genomic DNA and the homogeneity of the clonal neoplastic population. At this level, both high- and low-sensitivity analytical techniques detect HPV in > 90% of samples. Furthermore, the HPV-type specificity of PCR equals that of Southern blot hybridization, with HPV-16 identified in over 60% of cervical cancers (Riou *et al.*, 1990; van den Brule *et al.*, 1991; Higgins *et al.*, 1991b; Lörincz *et al.*, 1992). In penile cancers, 'high-risk' genital HPVs were detected in more than 48% of the biopsies by both techniques, with no major geographical differences in the detection frequency (McCance *et al.*, 1986; Tornesello *et al.*, 1992; Wiener *et al.*, 1992).

(*b*) *Expression of HPV proteins in the natural history of infection*

In benign lesions, late HPV proteins are expressed, with viral transcription patterns that vary by epithelial layer: weak expression of early genes occurs in the basal layers of low-grade cervical dysplasias induced by HPV-16 or HPV-33 and in some HPV-6- or HPV-11-induced condylomas; late genes are expressed in terminally differentiated keratinocytes of the superficial strata (Dürst *et al.*, 1992; Stoler *et al.*, 1992). Studies in HPV-16- and HPV-18-infected female renal transplant recipients demonstrate that,

following immunosuppression, antibodies to the late proteins decrease, whereas antibodies against early proteins E2, E4 and E7 significantly increase. This pattern suggests reactivation of latent virus (Lewensohn-Fuchs *et al.*, 1993). The regulation of gene expression is complex and is controlled by various cellular and viral transcription factors, different promoter usage, differential splicing, differential transcription termination and stability of mRNA.

In malignant lesions, integration of HPV DNA, generally concomitant with the disruption of *E2/E1* gene sequences, determines the major transcriptional changes. *E6* and *E7* are always transcribed actively in tumour cells (Schwarz *et al.*, 1985). The *E2* and/or *E1* disruption could lead to derepression of the P97 promoter. This, in turn, would modulate the expression of transforming genes and increase the transforming potential of HPVs (Lambert & Howley, 1988; Schiller *et al.*, 1989; Romanczuk & Howley, 1992; Jeon *et al.*, 1995).

(c) *Molecular mechanisms of transforming activity of HPV*

The transforming activity of HPV seems to be associated mainly with E6 and E7 open reading frames, which are consistently expressed in cervical cancers and cell lines derived from them (Smotkin & Wettstein, 1986; Hsu *et al.*, 1993). HPV-16 and HPV-18 *E6* and *E7* early genes, when expressed by a LTR promoter and transduced into cells by retroviral infection, immortalize human primary keratinocytes *in vitro* (Pirisi *et al.*, 1987; Schlegel *et al.*, 1988; Halbert *et al.*, 1991).

(i) *Intrinsic properties of high-risk HPV E6 and E7*

The HPV strains associated with malignant tumours (mainly HPV-16, -18, -31, -33, -35) are designated 'high-risk' HPV (IARC, 1995).

The E6 zinc finger protein of HPV-16 and HPV-18, like SV40 T antigen and adenovirus 5E1B, interacts specifically with the p53 tumour-suppressor protein. The p53–E6 complexes are then targeted to destruction through the ubiquitin-mediated proteolysis pathway (Scheffner *et al.*, 1990; Crook *et al.*, 1991). Thus it has been shown that expression of E6 in transfected cells abrogates a p53-controlled G1/S cell-cycle checkpoint (Kessis *et al.*, 1993; Foster *et al.*, 1994; Gu *et al.*, 1994; Canman *et al.*, 1995).

The E7 protein of high-risk HPV shares sequence homology with conserved regions 1 and 2 of the adenovirus E1a 243- and 289-amino-acid proteins. Like E1a, it binds to the product of the retinoblastoma gene, pRB (Dyson *et al.*, 1989; Münger *et al.*, 1989; Gage *et al.*, 1990). The RB protein is a phosphoprotein which, in its underphosphorylated form, appears to negatively regulate entry into the S-phase of the cell cycle; the initiation of S-phase is accompanied by pRB phosphorylation, via cyclin-dependent kinases. Binding of HPV E7 to pRB indirectly enhances transcription of several genes involved in cycle control, such as c-*myc*, c-*myb*, *cdc2*, DNA polymerase alpha, ribonucleotide reductase and thymidylate synthetase (Mudryi *et al.*, 1990; Nevins, 1992).

(ii) *Regulation of E6 and E7 expression*

Early gene expression is controlled by the long control region (LCR), extending over 400–900 bp, which may be considered to consist of three functional units. The 5' region,

adjacent to the L1 gene, contains the first E2 binding site as well as negative regulatory elements acting at the level of late mRNA stability (Kennedy *et al.*, 1991). The 3' segment contains a single E1 binding site (which identifies the origin of replication), an Sp1 transcription binding site, two E2 binding sites and the E6/E7 transcription promoter (Phelps & Howley, 1987; Swift *et al.*, 1987; Guis *et al.*, 1988). Between these two regions lies the HPV enhancer, the activity of which depends on cellular nuclear factors (Nakshatri *et al.*, 1990). In particular, the HPV-16 and HPV-18 enhancers contain recognition sites for cellular transcription factors such as *jun/fos* (Cripe *et al.*, 1990; Thierry *et al.*, 1992), nuclear factor I (NFI), transcription factor Sp1, activator protein AP1, glucocorticoid receptor and other papillomavirus enhancer-associated, but not yet characterized, factors (Chong *et al.*, 1990; Hoppe-Seyler & Butz, 1992). The activities of individual *cis*-acting elements contribute to the full enhancer activity. Published data suggest that HPV enhancer function depends on the cooperative interaction of multiple factors. Short segments of the enhancer have only a weak transactivating function. Frequently, recognition sites bind multiple proteins, and individual factors can interact with different recognition sequences (Chong *et al.*, 1990; Cripe *et al.*, 1990; Hoppe-Seyler & Butz, 1992; Thierry *et al.*, 1992).

Thus the expression of E6 and E7 could be enhanced by several mechanisms: mutational inactivation of *E2* or *E1* genes during HPV integration events; extracellular stimuli (growth factors, promoting agents, cytokines, etc.) via membrane receptors; or intracellular factors that bind the regulatory LCR, either directly or through activation of nuclear factors. For example, expression of HPV E6 and E7 can be modulated by the tumour promoter 12-*O*-tetradecanoylphorbol 13-acetate, which activates protein kinase C in the plasma membrane, eventually activating the nuclear transcription factor AP1 (Chan *et al.*, 1990).

4.4.2 *Interactions between HIV and HPV*

HIV is transmitted sexually (see Section 1.3.1). Although infection of squamous and colorectal epithelial cell lines or primary cultures has been reported (Adachi *et al.*, 1987; Tan *et al.*, 1993; Phillips *et al.*, 1994b), there is no convincing evidence of infection of epithelial cells by HIV *in vivo*.

Epithelial Langerhans' cells and related antigen-presenting cells in the layers beneath the mucosal epithelium are thought to be a major route of genital infection by HIV or SIV (Spira *et al.*, 1996). It is thus unlikely that the same cells *in vivo* will be co-infected by HIV and HPV. Even where HIV has been detected in CIN II biopsies, immunohistochemical evidence indicates that the HIV is localized to cells resembling lymphocytes or macrophages in the subepithelial stromal layer (Vernon *et al.*, 1994).

Infection and malfunction of Langerhans' cells could affect the local immune control of other HIV-infected cells. Furthermore, Spinillo *et al.* (1993) reported that counts of Langerhans' cells in CIN biopsies from HIV-infected women with CDC stage IV disease were significantly lower than those in CIN biopsies from HIV-negative matched controls.

(*a*) *Effects of HIV-related immunosuppression on HPV replication and HPV-associated anogenital lesions*

There are no experimental data addressing the effects of HIV-induced immunosuppression on HPV replication and transformation.

The epidemiological data reviewed in Section 2.3 suggest an increase in HPV genome copy numbers with immunosuppression. Higher HPV load may increase the probability of chromosomal integration of viral DNA and subsequent neoplastic events, as described above. Besides the increase in the number of HPV copies, HIV-infected immunosuppressed homosexual men as well as female transplant recipients often have multiple types of HPV (Palefsky *et al.*, 1992; Brown *et al.*, 1994b). However, the role of multiple HPV infection in the pathogenesis of anogenital neoplasia is unknown.

(*b*) *HIV Tat stimulation of cytokines and their role in genital lesions*

Cytokines have been shown to stimulate HPV-transformed epithelial cells. In particular, the pro-inflammatory cytokines IL-1α and TNFα, the expression of which is induced by Tat (Philippon *et al.*, 1994; Biswas *et al.*, 1995), inhibit proliferation of normal epithelial cells cultured from human cervix. However, they also significantly stimulate proliferation of cervical cell lines immortalized by transfection with HPV-16 or HPV-18 DNAs and of HPV-positive cell lines derived from cervical carcinoma. Growth stimulation by IL-1α or TNFα is accompanied by a 6–10-fold increase in RNA encoding amphiregulin, an epidermal growth factor receptor ligand (Woodworth *et al.*, 1995). However, whether this chain of events occurs *in vivo* is not known.

(*c*) *Possible effect of HIV-1 Tat on HPV E6/E7 expression*

Tornesello *et al.* (1991) reported that transfection of HIV-1 *tat* increased the expression of HPV-18 E7 in HeLa cells constitutively harbouring 10–20 copies of HPV-18. Expression of the HPV-16 LCR is also enhanced by HIV-1 Tat (Tornesello *et al.*, 1993; Vernon *et al.*, 1993). In addition, Tat increased the efficiency of E6/E7-mediated transformation of NIH 3T3 cells (Buonaguro *et al.*, 1994). Vogel *et al.* (1995) reported that transgenic mice carrying the HIV-1 *tat* gene express Tat protein in their keratinocytes. This is not sufficient to cause epidermal tumours, but is able to promote tumours after a single subthreshold dose of a carcinogenic initiator. Such tumour promotion has an effect additive to that of phorbol esters.

Although, as discussed above, HIV-1 and HPV are unlikely to co-infect the same cell, HIV-1 Tat has been shown to be released from infected cells (Frankel & Pabo, 1988). Extracellular Tat can be taken up by cervical epithelial cell lines (Frankel & Pabo, 1988; Frankel *et al.*, 1989; Ensoli *et al.*, 1993) and could thus allow the direct transactivation of HPV promoters.

In conclusion, both the immunosuppressive effect of HIV-1 infection and the secretion of HIV-1 Tat could promote the development of HIV-related precancerous anogenital lesions. Similar mechanisms might account for the increased incidence of other HPV-related and unrelated neoplasms in HIV infection.