British Journal of Dermatology (1988) **119**, 1–9.

T4/T8 ratio and absolute T4 cell numbers in different clinical stages of Kaposi's sarcoma in AIDS

P.SPORNRAFT*, M.FRÖSCHL*, J.RING*, M.MEURER*, F.-D.GOEBEL†, H.W.L.ZIEGLER-HEITBROCK‡, G.RIETHMÜLLER‡ AND O.BRAUN-FALCO*

* Dermatologische Klinik und Poliklinik, † Medizinische Poliklinik and ‡ Institut für Immunologie, Ludwig Maximilians Universität München, F.R.G.

Accepted for publication 30 December 1987

SUMMARY

Thirty-seven men (36 homosexual or bisexual and one heterosexual) with epidemic Kaposi's sarcoma and underlying HIV infection were followed up over a period of up to 32 months. Fourteen patients (38%) died, with a median survival time of 7·2 months after the diagnosis of AIDS. Seventeen patients (46%) presented with one or more opportunistic infections, mostly *Pneumocystis carinii* pneumonia. Eighteen patients (49%) had lymphadenopathy syndrome according to the definition of the CDC. Using the Laubenstein-classification of Kaposi's sarcoma, all patients either remained stable or deteriorated, improvement was never observed. Absolute T4 lymphocyte counts and the T4/T8 ratio were not related to the disease stage. With the onset of B symptoms (systemic symptoms), however, the absolute T4 numbers and the T4/T8 ratio markedly decreased. Delayed type hypersensitivity also showed no relationship to the clinical stages of Kaposi's sarcoma. Thus, the clinical progression of Kaposi's sarcoma lesions seems to be largely independent of the immunological parameters investigated. However, the onset of B symptoms was observed to be related to changes in immune status.

Disseminated Kaposi's sarcoma (epidemic type) is one of the main manifestations of the acquired immune deficiency syndrome (AIDS) and occurs almost exclusively among homosexual men.[1–3] Patients with epidemic and also with classical Kaposi's sarcoma seem to have a greater susceptibility to other malignancies including non-Hodgkin's lymphomas and leukaemias,[2,4,5] pointing to a possible genetic factor.

The appearance of Kaposi's sarcoma has also been linked to infections with several viruses, especially cytomegalovirus and Epstein-Barr virus which are known to be capable of transforming lymphocytes *in vitro*.[4,6,7] Cytomegalovirus infection is also known to cause immunosuppression in both humans and mice.[8]

Furthermore, it has been observed that patients receiving immunosuppressive therapy, for example renal transplant recipients, showed an increased incidence of epidemic Kaposi's sarcoma.[2,4] These findings suggest a possible relationship between immunosuppression and the onset of this otherwise unusual tumour. AIDS patients with Kaposi's sarcoma only, compared with those with Kaposi's sarcoma plus opportunistic infections, present with a better clinical and immunological status and have a greater life expectancy.[4,9] Considering the different clinical stages of Kaposi's sarcoma, we hypothesized that with progression of the disease the immunological parameters would be expected to continue to deteriorate.

We have studied T4 and T8 cells and delayed type hypersensitivity in patients with different clinical stages of Kaposi's sarcoma.

METHODS

Patients

Thirty-six Caucasian homosexual or bisexual men, and one heterosexual man, with epidemic Kaposi's sarcoma and underlying HIV infection were examined. The mean age was 38 years (range 25–65 years). The mean observation time was 8·1 months and ranged from 1 to 32 months (up to the end of February 1987).

Diagnosis of Kaposi's sarcoma was confirmed by biopsy. The onset of AIDS (if the patient did not show any previous opportunistic infections) was determined by the date of the first biopsy revealing Kaposi's sarcoma, even if the patient had given a history of purple blue lesions before this. Diagnosis of opportunistic infections according to the definition of the Centers for Disease Control (CDC), Atlanta, U.S.A. was proven by bronchoscopy and bronchial lavage (*Pneumocystis carinii* pneumonia), oesophago-gastro-duodenoscopy (*Candida* oesophagitis) and by electron microscopy using a negative contrast technique (ulcerating herpes simplex). If this latter procedure failed to show virus-like particles the diagnosis was made clinically on typical morphological features. Toxoplasmosis and disseminated cytomegalovirus infection observed in one patient were diagnosed at autopsy.

Clinical staging of Kaposi's sarcoma

The clinical stages of Kaposi's sarcoma were determined using the classification of Krigel, Laubenstein and Muggia[10] with slight modifications: Grade I cutaneous, locally indolent; Grade II cutaneous, locally aggressive with or without palpable regional lymph nodes; Grade III mucocutaneous with or without palpable regional lymph nodes; Grade IV visceral; A no systemic signs or symptoms; B systemic signs: nightsweats, fever, weight loss of more than 10%.

Human immunodeficiency virus (HIV) serology

As a screening test, an enzyme-linked immunosorbent assay (ELISA) was used (Du Pont de Nemours, Bad Nauheim, Germany). All results were confirmed by Western blot or immunofluorescence or both.

Immunological parameters

Lymphocyte subpopulations. Peripheral blood mononuclear cells were isolated from heparinized blood by Ficoll-Hypaque density gradient separation and cells were stored in liquid nitrogen. For immunofluorescence, cells were reacted with monoclonal antibodies of the CD4 cluster (helper cells, MT 151,)[11] and of the CD8 cluster (cytototoxic/suppressor cells, MT

811,)[11] at saturating concentrations for 30 min on ice. After two washes the cells were incubated with goat anti-mouse Ig FITC (Tago, Burlingame, California, U.S.A.) for another 30 min and after final washes they were analysed under a Leitz orthoplan fluorescence microscope with PLoem epi-illumination. In each sample 150–200 cells were analysed. The percentage of positive cells was used to calculate the T4/T8 ratio and the absolute counts of lymphocytes and monocytes in the peripheral blood. The ratio of T helper to T suppressor/cytotoxic cells and the absolute numbers of T helper cells were determined in 29 and 24 patients, respectively. In most patients several examinations were carried out during the course of the study.

Delayed type hypersensitivity. For evaluation of cellular immunity an intradermal test with recall antigens was performed using the 'Multitest Merieux' system providing seven microbial antigens and one control (glycerol). The tests were read after 48 h and the diameters of the positive skin reactions measured. The patients were divided into three categories on the basis of the sum of the diameters of the skin test reactions: Anergy (0–1 mm), hypoergy (2–9 mm), normoergy (> 10 mm).

Statistical analysis

The groups were compared using a Mann Whitney U test.

RESULTS

Clinical data

All patients fulfilled the clinical criteria of the CDC for AIDS, together with positive HIV serology including confirmatory tests.

Seventeen of the 37 patients (46%) presented with one or more opportunistic infections either before or after the diagnosis of Kaposi's sarcoma. Most developed *Pneumocystis carinii* pneumonia and presented at the same time or later with ulcerating herpes simplex infection and *Candida* oesophagitis. Additionally, many patients showed various skin infections including herpes zoster infection, non-ulcerating herpes simplex, molluscum contagiosum and multiple infections with human papilloma viruses such as verrucae vulgares or condylomata acuminata. Oral, genital and intestinal candidosis was also detected (Table 1). Eighteen patients (49%) showed signs of lymphadenopathy syndrome according to the definition of the CDC, together with Kaposi's sarcoma. Almost all patients (35) had a past history of other sexually transmitted diseases including syphilis, gonorrhoea and hepatitis B.

During the period of observation 14 patients died (38%); seven of these had developed opportunistic infections. In four cases the cause of death remained unclear because autopsy was refused. One patient committed suicide after the diagnosis of *Pneumocystis carinii* pneumonia was made and another, a renal transplant recipient (HIV-positive, homosexual), died of acute renal failure. In three patients death seemed to be directly caused by Kaposi's sarcoma; two died with gastrointestinal obstruction due to Kaposi's sarcoma and one patient died of obstructive, extremely exophytic lesions of the tumour in the pharynx and larynx. The average time of survival after the diagnosis of AIDS was 7·2 months.

All patients who later died had suffered from B symptoms at some time. This corresponds with the finding that seven of these patients had presented with documented opportunistic infections. On the other hand all patients without B symptoms (classified as A) were still alive at the end of the observation period. Thus, the median survival of 12 patients without systemic symptoms was 100% at 32 months, whereas out of 25 patients with B-symptoms 14 (56%) died within the same time period.

TABLE 1. Clinical findings in 37 patients with Kaposi's sarcoma and AIDS

	No. of patients	%
Opportunistic infections		
Pneumocystis carnii pneumonia	12	32
Ulcerating herpes simplex	7	19
Candida oesophagitis	3	8
Atypical mycobacteriosis, disseminated	1	3
Toxoplasmosis*	1	3
Cytomegalovirus infection, disseminated*	1	3
Other infections with skin manifestions		
Herpes zoster infection	3	8
Herpes simplex (non ulcerating)	12	32
Verruca vulgaris	6	16
Condyloma acuminata	2	5
Molluscum contagiosum	5	14
Candidosis, oral, genital or intestinal	24	65

* Found at autopsy in a patient who developed *Pneumocystis carnii* pneumonia.

Staging of patients according to the Laubenstein classification,[10] is listed in Table 2. From the first examinations at the beginning of the study over a period of a maximum of 32 months, 25 patients remained stable, while 12 patients deteriorated. Changes from one stage to another occurred only for the worse; improvement in stages was never observed. Almost all patients with opportunistic infections suffered from B symptoms.

There were no significant differences in the T4/T8 ratios or absolute T4 counts between patients in the A stage of each grade and those in the B stage (Table 3). This is probably because

TABLE 2. Classification of Kaposi's sarcoma in 37 patients with AIDS according to the Laubenstein classification[10]

		No. of patients at end of observation period (1–32 months)								
		Stage								With opportunistic infection
Disease stage	No. of patients at beginning of observation period	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB	
IA	3	2	1	...	...	...	...	...	...	...
IB	0	...	...	...	...	...	...	...	...	...
IIA	9	...	...	6	2	...	1	...	...	4
IIB	7	...	...	...	6	...	1	...	...	4
IIIA	8	...	...	...	...	3	4	...	1	3
IIIB	6	...	...	...	...	...	4	...	2	5
IVA	2	...	...	...	...	...	...	2	...	...
IVB	2	...	...	...	...	...	...	...	2	1
Total	37	2	1	6	8	3	10	2	5	17

TABLE 3. T4/T8 ratio and absolute T4 lymphocyte counts in different clinical stages of Kaposi's sarcoma in AIDS

	Disease stage							
	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
T4/T8 ratio*	0·53 ($n=3$)	0·30 ($n=1$)	0·57 ($n=8$)	0·38 ($n=7$)	0·46 ($n=7$)	0·19 ($n=3$)	0·57 ($n=2$)	0·30 ($n=1$)
absolute T4 counts (cells/μl)*	364 ($n=3$)	105 ($n=1$)	330 ($n=5$)	136 ($n=5$)	213 ($n=6$)	139 ($n=3$)	288 ($n=2$)	157 ($n=1$)

* Overall mean for each disease stage calculated from individual patient means.

of the small numbers of patients, because lower T4/T8 ratios and absolute T4 counts were found in B stage patients than in A stage patients at each grade (Figs 1 and 2). Comparison, however, of the mean values for all patients in stage A with all those in stage B showed significantly higher absolute T4 counts in the patients without systemic symptoms (stage A) ($P=0·02$). The difference in the T4/T8 ratio was not significant ($P=0·2$).

Delayed type hypersensitivity was examined in 27 patients using an intradermal test with recall antigens ('Multitest Merieux'). At the beginning of the observation period 16 patients (59%) were anergic, five (19%) showed diminished cutaneous responses and six (22%) showed normal responses. The cutaneous responses are listed according to disease stage in Table 4, which also indicates the numbers of patients whose category of skin reactivity changed during observation period. Again, only progression towards anergy was found; no changes occurred in the other direction. There was no apparent relationship of the degree of cutaneous response with any of the disease grades nor with A or B stages.

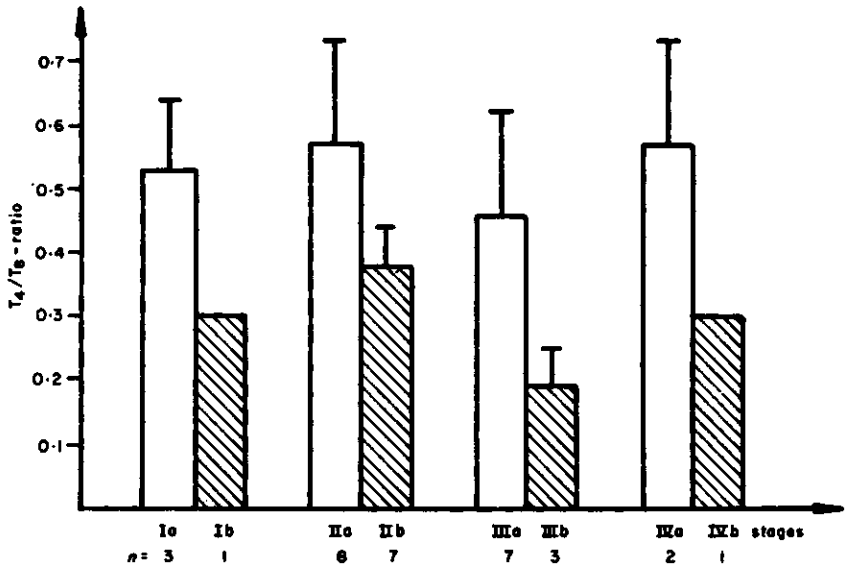

FIGURE 1. T4/T8 ratios at different clinical stages of Kaposi's sarcoma in AIDS. Bars indicate means ± SEM.

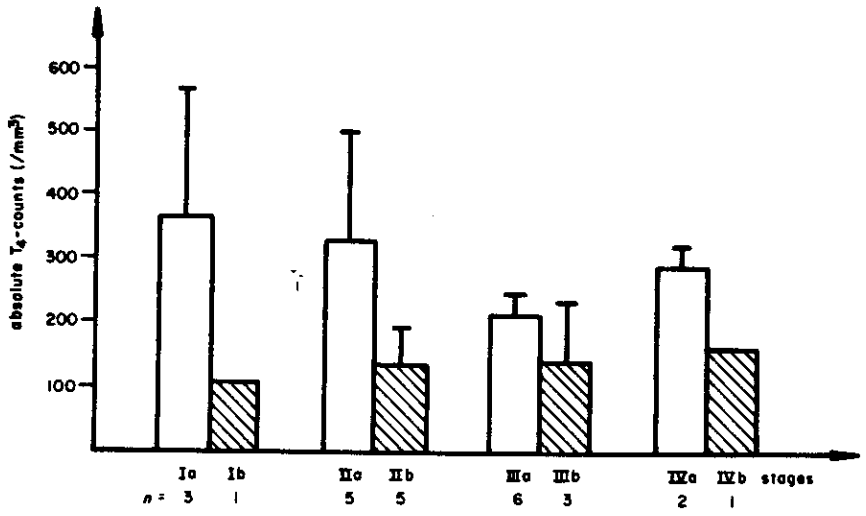

FIGURE 2. Absolute T4 lymphocyte counts in different clinical stages of Kaposi's sarcoma in AIDS. Bars indicate means ± SEM.

TABLE 4. Delayed type cutaneous reactions at different clinical stages of Kaposi's sarcoma in AIDS

Skin reactivity*	No. of patients in clinical stages							
	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB
Normergy	1	···	4(2) ↓	···	···	1(1) ↓	···	···
Hypoergy	···	···	···	2(1) ↓	1	1	···	1
Anergy	2	···	4	5	3	1	1	···
Total	3	0	8	7	4	3	1	1

* Sum of positive skin reactions: > 10 mm = normergy; 2–9 mm = hypoergy and 0–1 mm = anergy. Arrows indicate numbers of patients who changed category during the period of the study.

When skin reactivity was plotted against T4/T8 ratios measured at the same time, there was no significant correlation (Fig. 3). However, there was a trend towards a positive correlation between delayed type hypersensitivity and absolute T4 counts ($r = 0{\cdot}59$; Fig. 4).

DISCUSSION

The results of this study demonstrate clear differences in the cellular immune parameters T4 numbers, T4/T8 ratio and intradermal delayed type hypersensitivity, between subgroups of patients with Kaposi's sarcoma and AIDS. There was no apparent relationship between these

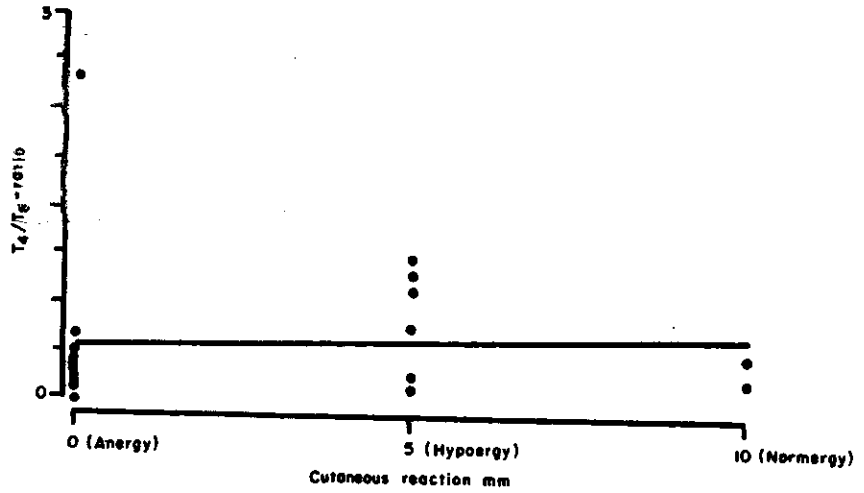

FIGURE 3. Relationship between delayed type cutaneous reactions (sum of diameters of positive reactions in mm) and T4/T8 ratio in patients with Kaposi's sarcoma in AIDS. ($r=0{\cdot}084$).

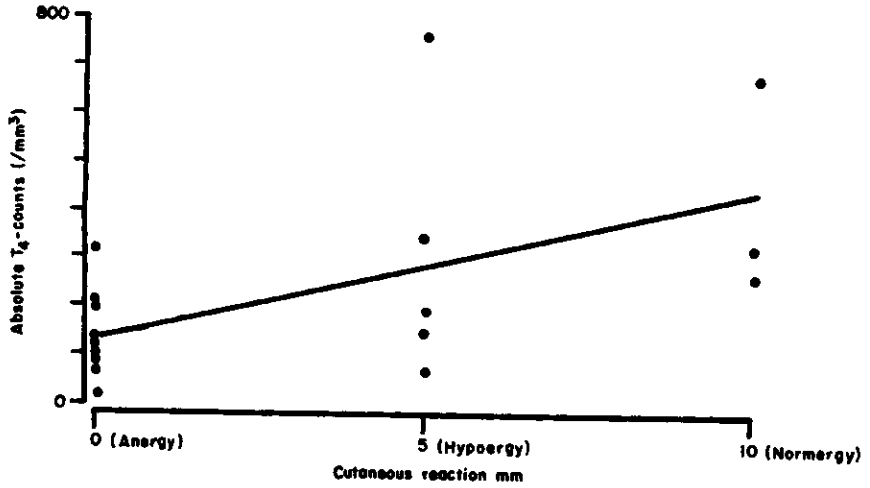

FIGURE 4. Relationship between delayed type cutaneous reactions (sum of diameters of positive reactions in mm) and absolute T4 counts in patients with Kaposi's sarcoma in AIDS. ($r=0{\cdot}585$).

immune parameters and the extent of Kaposi's sarcoma lesions as graded by the Laubenstein classification. Patients suffering from B symptoms, however, had lower T4 values than patients in the A subgroup, irrespective of the extent of the lesions (including visceral involvement). Thus, widespread Kaposi's sarcoma in AIDS can occur with almost normal cellular immune parameters.

Kaposi's sarcoma develops in about 30% of all AIDS patients and affects almost exclusively homosexual men as was the case in our patients. It has been observed that AIDS patients with Kaposi's sarcoma alone have a better prognosis and a longer life expectancy than those who have opportunistic infections also.[9,12] Krigel[13] reported an 80% survival at 28 months in patients with epidemic Kaposi's sarcoma (EKS) compared with a survival rate of less than 20% in patients with EKS and concomitant opportunistic infections. Another early study showed

similar results.[12] The classification of Kaposi's sarcoma by Krigel, Laubenstein and Muggia[10] includes A (no systemic symptoms) and B symptoms (presence of systemic signs). In the present study 25 patients (65%) had developed B symptoms within 32 months, of whom 17 presented with opportunistic infections. Of the 25 patients with B symptoms, 14 died within 32 months at least seven of these having suffered opportunistic infections. Thus, the survival rate of patients with opportunistic infections. Of the 25 patients with B symptoms, 14 died within 32 months, at least seven of these having suffered opportunistic infections. Thus, the survival rate of patients an earlier study: of 15 patients with EKS, those without systemic signs had a survival rate of 100% whereas those with B symptoms had only 50% survival at 28 months.[14] These results suggest that the onset of systemic symptoms represents an important prognostic factor in the course of EKS in AIDS.

However, the extent of the skin lesions, including gastro-intestinal involvement, did not have any prognostic value. Gastrointestinal tract involvement has been described in about 45% of cases of EKS[10,15] and was not related to the survival rate.[15]

These clinical findings are in agreement with the immunological findings; patients with EKS alone seem to be less immunosuppressed than those presenting with opportunistic infections also.[16] Among the many immunological abnormalities described in HIV infection[9,11] the T4/T8 ratio, absolute T4 numbers and intradermal reactivity are most widely used. The T helper/T suppressor (T4/T8) ratio and the absolute T4 numbers are comparatively higher in patients with EKS as the only manifestation of AIDS.[9,17] The lowered T4/T8 ratio could also be due to a relative increase in the numbers of T8 suppressor/cytotoxic lymphocytes which is seen in other viral infections but also in early stages of HIV infection.[11,17-19] Therefore, the absolute T4 lymphocyte count seems to be a more reliable measure of immunosuppression in AIDS.[17,19]

In the present study patients with systemic symptoms (B stages) showed a marked reduction of the T4/T8 ratio and absolute T4 numbers compared with the patients with no systemic signs (A stages). When results for patients in all clinical stages were analysed together there was a significant difference between A and B patients with respect to absolute T4 numbers, but not the T4/T8 ratio. Thus the decrease in T4 lymphocyte numbers shows closer relationship with onset of B symptoms in EKS than does the decrease in the T4/T8 ratio.

Additionally, delayed type hypersensitivity, as tested with recall antigens, was found to be depressed already at the first examination in many patients (59%). This corresponds with previous findings in patients with EKS[2] and with lymphadenopathy-syndrome.[20] Thus, cutaneous anergy seems to be an early indicator of immunological dysfunction. However, cutaneous responses can also be intact in the early stages of the syndrome.[21]

Finally, concerning the clinical classification of Kaposi's sarcoma, about one third (12 of 37) of the patients studied showed progression of the disease and 38% died during the period of observation. Other authors give a higher percentage of progression over similar time periods. Brodt *et al.*[22] also observed patients with opportunistic infections. These authors stated that the onset of all the manifestations of HIV infection depend on the condition of the patient at his first examination, the degree of immunosuppression and on the period of observation.

In the present study there was no correlation between delayed type hypersensitivity and the T4/T8 ratio, but there was a tendency towards a relationship with T4 numbers.

Thus, a defect in cellular immunity appears to be a prerequisite for Kaposi's sarcoma. However, progression of the lesions seems to be independent of the degree of immunodeficiency, at least with respect to T cell subpopulations and skin reactivity. However, more exhaustive study of T lymphocyte subpoplations, for example, expansion by two colour fluorescence,[23] might reveal a relationship with disease stage.

ACKNOWLEDGMENTS

We thank Professor R.M.Eder, Pathology Institute, Ludwig Maximilians University for performing the autopsy and Dr L.G. Gürtler and Professor F. Deinhardt, Max von Pettenkofer Institute, Ludwig Maximilians University for the HIV serology.

REFERENCES

1 Braun-Falco O. Klinik von AIDS. Aus der Sicht des Dermatologen. *Münch Med Wochenschr* 1986; 128: 270–5.
2 Krigel RL, Friedman-Kien AE. Kaposi's sarcoma in AIDS. In: *AIDS: Etiology, Diagnosis, Treatment and Prevention* (de Vita VT, ed). JV Lippincott, 1985; 185–211.
3 Marmor M. Epidemic Kaposi's sarcoma and sexual practices among male homosexuals. In: *AIDS. The Epidemic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 291–6.
4 Friedman-Kien AE, Ostreicher R. Overview of classical and epidemic Kaposi's sarcoma. In: *AIDS. The Epidemic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 23–31.
5 Warner LC, Fisher BK. Cutaneous manifestations of the acquired immunodeficiency syndrome. *Int J Dermatol* 1986; 25: 337–50.
6 Drew WL, Conant MA, Miner RC *et al.* Cytomegalovirus and Kaposi's sarcoma in young homosexual men. *Lancet* 1982; 2: 125.
7 Fenaglio CM, McDougall JK. The relationship of cytomegalovirus to Kaposi's sarcoma. In: *AIDS. The Epidemic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 229–336.
8 Rubinstein P, Walker M, Mollen N *et al.* Immunogenetic aspects of epidemic Kaposi's sarcoma in homosexual men. In: *AIDS. The Epidemic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 139–46.
9 Lane HC, Fauci AS. Immunologic abnormalities in the acquired immune deficiency syndrome. In: *AIDS. The Epidermic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 147–52.
10 Krigel RL, Laubenstein LJ, Muggia FM. Kaposi's sarcoma: A new staging classification. *Cancer Treat Rep* 1983; 67: 531–4.
11 Rieber EP. Störungen der Immunantwort bei LAS und AIDS. *Münch Med Wochenschr* 1986; 128: 463–6.
12 Volberding P. Therapy of Kaposi's sarcoma in AIDS. *Semin Oncol* 1984; 11: 60–7.
13 Krigel RL. Prognostic factors in Kaposi's sarcoma. In: *AIDS. The Epidemic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 69–72.
14 Brunner R, Ring J, Braun-Falco O. Kaposi-Sarkom. Behandlungsverfahren im Rahmen des erworbenen Immunmangelsyndroms (AIDS). *Münch Med Wochenschr* 1986: 128: 467–9.
15 Horowitz L, Stern JO, Segarra S. Gastrointestinal manifestations of Kaposi's sarcoma and *AIDS. In: AIDS. The Epidemic of Kaposi's Sarcoma and Opportunistic Infection* (Friedman-Kien AE, Laubenstein LJ, eds). New York: Masson Publishing, 1984; 235–9.
16 Vadhan-Raj S, Wong G, Gnecco C *et al.* Immunological variables as predictors of prognosis in patients with Kaposi's sarcoma and the aquired immunodeficiency syndrome. *Cancer Res* 1986; 46: 417–25.
17 Bowen DL, Lane HC, Fauci AS. Immunologic features of AIDS. In: *AIDS: Etiology, Diagnosis, Treatment and Prevention* (de Vita VT, ed). JV Lippincott, 1985; 89–109.
18 Cooper DA, Maclean P, Finlayson R *et al.* Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. *Lancet* 1985; i, 537–40.
19 Pichler WJ. Zellulär-immunologische Abklärung bei HIV (LAV/HTLV-III)-Infektion. *Schweiz Med Wochenschr* 1986; 116: 1506–13.
20 Metroka CE, Cunningham-Rundles S, Pollack MS *et al.* Generalized lymphadenopathy in homosexual men. *Ann Intern Med* 1983; 99: 585.
21 Siegal FP. Normal delayed type skin reactions in early stages of acquired cellular immunodeficiency. *N Engl J Med* 1982; 307: 184.
22 Brodt HR, Helm EB, Werner A *et al.* Spontanverlauf der LAV-/HTLV-III-Infektion. Verlaufsbeobachtungen bei Personen aus AIDS-Risikogruppen. *Dtsch Med Wochenschr* 1986; 111: 1175–80.
23 Ziegler-Heitbrock HWL, Schramm W, Stachel D *et al.* Expansion of a minor subpopulation of peripheral blood lymphocytes (T8+/Leu7+) in patients with hemophilia. *Clin Exp Immunol* 1985; 61: 633–41.