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Research Article

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Deficient Autologous Mixed Lymphocyte Reaction in Kaposi's Sarcoma Associated with Deficiency of Leu-3+ Responder T Cells

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ABSTRACT Autologous mixed lymphocyte reaction (AMLR) and T cell subsets defined with monoclonal antibodies were analyzed in the peripheral blood of homosexual males with Kaposi's sarcoma (KS). All seven patients demonstrated decreased AMLR ($P < 0.001$) when compared with age- and sex-matched simultaneously studied controls. These patients also showed decreased proportions of Leu-3+ (helper/inducer phenotype) and an increase in the proportion of Leu-2+ (suppressor/cytotoxic phenotype) T cells. Leu-3+ T cells were purified from two patients by depleting Leu-2+ T cells in complement-dependent cytotoxicity. Leu-3+ T cells from both patients demonstrated poor proliferative response in the AMLR. In allogeneic MLR, patients' T cells were poor responders and their non-T cells were poor stimulators against healthy controls. This study demonstrates deficiency of both AMLR and allogeneic MLR in patients with KS. The decreased AMLR is associated with qualitative and functional deficiency of Leu-3+ responder T cells. Whether the functional deficiency of Leu-3+ responder T cells in the AMLR is a general phenomena or a feature of a subset of patients with KS remains to be determined.

INTRODUCTION

Kaposi's sarcoma (KS) is a neoplasm of multicentric origin characterized by tumors with vascular and fibroblastic elements. It is a rare disease; however, increased incidence of KS has been found in patients after renal transplantation (1) and in homosexuals (2). The course of disease ranges from slow and indolent to rapid and fulminant with metastatic dissemination. Recent studies have demonstrated a number of immune defects in homosexual males (3–5). These subjects have high incidence of infections with *Pneumocystis carinii* and viruses (3–5). Association of cytomegalovirus (CMV) infection and KS has been documented (6). In autologous mixed lymphocyte reaction (AMLR), T cells proliferate upon stimulation with autologous non-T cells with two cardinal features of an immune response, the memory and specificity (7, 8). In AMLR, generation of helper and suppressor functions have been described (9–11). Recently it has been shown that T cells with helper/inducer phenotype (OKT4+, Leu-3+) respond in AMLR and OKT8+ and Leu-2+ (suppressor/cytotoxic phenotype) T cells either do not respond or respond poorly in AMLR (12–15). In this investigation we have examined AMLR in seven patients with KS and simultaneously studied T cell subsets defined with monoclonal antibodies. Our study shows a marked decrease in AMLR in KS associated with deficiency of the Leu-3+ T cells.

METHODS

Seven patients with KS and seven age- and sex-matched healthy controls were simultaneously studied. The characteristics of these patients are shown in Table I. None of these patients received any treatment at the time of the study. All patients had active form of KS as manifested by skin manifestation, lymphadenopathy, and gastrointestinal involvement. Fluorescein-conjugated monoclonal antibodies (Leu-1, Leu-2a, and Leu-3a) were purchased from Becton-Dickinson & Co. (Mountain View, CA).

Mononuclear cells from fresh heparinized (20 U/ml) peripheral venous blood were separated on Ficoll-Hypaque density gradient. Mononuclear cells were washed three times with Ca^{++}- and Mg^{++}-free Hanks' balanced salt solution

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[1] *Abbreviations used in this paper*: AMLR, autologous mixed lymphocyte reaction; CMV, cytomegalovirus; CTL, cytotoxic T lymphocytes; HSV, herpes simplex virus; KS, Kaposi's sarcoma; MHC, major histocompatibility complex; NK, natural killer.

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TABLE I
Autologous MLR in Patients with KS and Simultaneously Studied Healthy Controls

Patient	Age/sex	Absolute lymphocyte counts	Genital herpes	PC§	HSV1 Titers‡	CMV titers*		CMV‖	T cell subsets			
						IgM	IgG		Leu-1	Leu-2a	Leu-3a	Leu-3a/ Leu-2a
		/mm³							%			
1	33/M	1,050	−	+	Anticomp	1/64	1/28	+	39	40	28	0.70
2	35/M	1,020	−	−	ND	1/64	1/1024	+	42	30	31	1.03
3	41/M	2,400	−	+	1/64	1/32	1/256	−	49	71	28	0.39
4	35/M	3,264	−	−	1/8	1/8	1/516	−	52	65	25	0.38
5	38/M	175	−	−	1/16	1/64	1/64	−	31	62	29	0.46
6	43/M	1,349	+ (rectal)	+	1/64	1/8	1/1024	−	48	49	32	0.65
7	41/M	1,016	−	−	1/64	1/32	1/64	−	50	47	30	0.63
Mean±SD	—	1,467±1,029	—	—	—	—	—	—	44.4±7.5	52.0±14.7	29.0±2.3	0.6±0.2
Controls(7)	37±3/M	2,242±642	—	—	—	—	—	—	68.4±4.4	29.2±2.3	56.6±5.6	1.9±0.2

* CMV antibody titers were assayed by ELISA (enzyme-linked immunosorbent assay). Serial serum samples did not show any rise in IgM titers indicating absence of active infection.

‡ HSV, herpes simplex virus. Antibody titers were assayed by complement fixation. These low titers show absence of active disease.

§ PC = *P. carinii*, diagnosis made by lung biopsy or by bronchial alveolar lavage.

‖ CMV was isolated from urine. Anticomp, anticomplementary; ND, not done.

(HBSS, Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) and resuspended in HBSS at a concentration of 3–4 × 10^6 cells/ml. The viability of cells was >98% by trypan blue dye exclusion. Analysis of T cells and T cell subsets was performed with Leu-1 (pan-T), Leu-2a (suppressor/cytotoxic phenotype), and Leu-3a (helper/inducer phenotype) monoclonal antibodies by a fluorescent-activated cell sorter (FACS, Becton Dickinson & Co.).

T and non-T cells were purified by rosetting mononuclear cells with neuraminidase-treated sheep erythrocytes and separating rosetted T cells from non-rosetted non-T cells on Ficoll-Hypaque gradient (12). Both T and non-T cells were washed three times with HBSS and resuspended in RPMI 1640 containing 20 mM of Hepes buffer, 10% human AB serum, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 2 mM of L-glutamine (Gibco Laboratories), hereafter referred to as complete medium. Cells were adjusted to a concentration of 1 × 10^6/ml. T cells obtained were >95% pure as determined by rosette formation with sheep erythrocytes and reaction with pan-T cell monoclonal antibody and lack of cells with detectable surface immunoglobulin. Monocyte contamination in T cell fractions as determined with antimonocyte monoclonal antibodies (Bethesda Research Laboratories, Bethesda, MD) was <2%. The non-T cell fractions were >60% surface Ig^+ B cells, 20% monocytes (react with antimonocyte monoclonal antibody), 15–20% non-T, non-B cells (null cells), and 4% T cells. The viability in both fractions was >95% as determined with trypan blue dye exclusion.

Mixed lymphocyte cultures were performed in triplicates in round-bottom microtiter plates (Nunc, Denmark) at 37°C in a humidified 50% CO_2 atmosphere. Each well consisted of 200 μl of culture medium containing 1 × 10^5 responder T cells and 1 × 10^5 irradiated non-T cells (3,000 rad from a cesium source) as stimulators. Cultures were terminated on days 5, 6, and 7. Cultures were pulsed with 2 μCi/well of [^{3}H]thymidine (New England Nuclear, Boston, MA) for the final 18 h of culture. The cultures were harvested onto a glass fiber filter strip by automated multiple sample harvester (Flow Laboratories, Inc., McLean, VA). The thymidine incorporation was determined by a liquid scintillation counter. The data are expressed for the peak proliferative response in Δ counts per minute, defined as the difference in counts per minute from triplicates of cultures containing both responder T cells and stimulator non-T cells from the sum of counts per minute from triplicates of responder T cells and stimulator non-T cells when each were cultured alone. Statistical analysis was done by using the *t* test.

$Leu-3^+$ T cells were purified from two patients with KS and two healthy controls to examine the response of separated population in AMLR. $Leu-3^+$ cells were purified by depleting $Leu-2^+$ T cells with Leu-2 monoclonal antibody and complement. This procedure was repeated twice. Leu-3-enriched T cells were >95% $Leu-3a^+$ and contained <3% $Leu-2a^+$ T cells by fluorescent-activated cell sorter analysis.

RESULTS

Data of T cell subsets are shown in Table II. The proportion of total T cells as defined with Leu-1 antibody in KS (mean±SD, 44.4±7.5) was significantly lower ($P < 0.01$) than that of simultaneously studied controls (mean±SD, 68.4±4.4). The proportions of $Leu-2a^+$ T cells was significantly increased ($P < 0.001$) in patients with KS (mean±SD, 52.0±14.7) as compared with the values for normal controls (mean±SD, 29.2±2.3). In contrast, the proportion of $Leu-3a^+$ T cells in KS (mean±SD, 29.0±2.3) was significantly lower ($P < 0.001$) than that observed in healthy controls (mean±SD, 56.6±5.6). This resulted in a significantly lower ($P < 0.001$) ratio of Leu-3/Leu-2 cells in KS

Table II
AMLR in Patients with Kaposi's Sarcoma in Simultaneously Studied Controls

Subjects	[^{3}H]Thymidine incorporation: Patients: Background	Patients: AMLR*	Controls: Background	Controls: AMLR
		cpm		
1	507	788	579	12,078
2	430	1,213	241	3,568
3	458	2,302	341	8,432
4	616	967	246	7,642
5	543	822	476	10,402
6	518	1,237	646	6,406
7	982	1,615	784	9,328
Mean±SD	578±187	1,278±584‡	473±208	8,265±2,773

* Counts expressed as Δ counts per minute after subtracting background counts from total counts of AMLR.
‡ $P < 0.001$, as compared with controls.

(mean±SD, 0.6±0.3) when compared with controls (mean±SD, 1.9±0.2). In the AMLR, patients demonstrated significantly ($P < 0.001$) poor proliferative response (mean±SD, 1,278±534) when compared with simultaneously studied controls (mean±SD, 8,265±2,773).

Results of the analysis of AMLR between Leu-3a-enriched T cells (after depletion of Leu-2$^+$ cells) and non-T cells from two patients with KS and two normal controls are shown in Table III. AMLR in patients was significantly lower ($P < 0.01$) than in the controls. No significant enhancement ($P > 0.5$) was observed in [^{3}H]thymidine incorporation between unseparated T cells and Leu-3a-enriched T cells in AMLR. This suggests that increased suppressor cell activity is not responsible for decreased AMLR. Data on allogeneic MLR are shown in Table IV. Patient T cells responded poorly to control non-T cells as well as patient non-T cells stimulated control T cells subnormally when compared with allogeneic MLR between healthy controls.

No correlation was observed between any of the above immunological tests and titers of CMV or HSV antibodies, presence of *P. carinii*, activity of KS, or isolation of CMV virus (Table I).

Table III
AMLR between LEU-3a-Enriched T Cells and Non-T Cells in KS and Normal Control

	[^{3}H]Thymidine incorporation, Responder T cells: Unseparated T: Background	Unseparated T: AMLR	Leu-3a$^+$ T‡: Background	Leu-3a$^+$ T‡: AMLR
		cpm		
Patient 6	518	1,237*	428	1,880*
Control 6	646	6,406	524	8,420
Patient 4	732	1,240*	434	2,042*
Control 4	346	8,642	440	11,420

* P value when compared with control is <0.01.
‡ Leu-3a$^+$ T cells were obtained by double cycle depletion of Leu-2$^+$ T cells by complement-dependent cytotoxicity.

DISCUSSION

In this study we have demonstrated decreased AMLR in patients with KS. The deficiency of AMLR in each patient was associated with a decrease in the proportion of Leu-3a$^+$ T cells. Our patients were in their 3rd and 4th decades. It is not known whether similar abnormalities of T cell subsets and/or the AMLR would be present if patients were in the 2nd and 3rd decades. Moody et al. (16) studied AMLR and T cell subsets in young and aging subjects; but they did not present data for the two decades separately. However, in the present study we simultaneously studied age-matched controls. We and others (13–15) have demonstrated that in AMLR the major responding T cells are Leu-3$^+$ and OKT4$^+$. Therefore, deficiency of AMLR could be due to quantitative deficiency of Leu-3$^+$ T cells. However, possibility of a functional defect of Leu-3$^+$ T cells, a defect of stimulating non-T cells, serum inhibitory factor, or suppressor T lymphocytes should be entertained. To address the latter possibility we examined AMLR in purified Leu-3a$^+$ T cells obtained by depleting Leu-2$^+$ T cells from two patients. AMLR was still markedly decreased when compared with controls. These results show that decreased AMLR was not due to suppressor T lymphocytes. Whether this defect of Leu-3$^+$ responder T cells is present in all patients with KS or is a feature of a subset of patients, requires similar studies in a large number of patients. Serum inhibitory factor appears unlikely because cells were extensively washed at least six times and cultures

Table IV
Allogeneic MLR between T and Non-T Cells from Patients with KS and Simultaneously Studied Healthy Controls

Responder T cells	Stimulator non-T cells	Mean±SD
		cpm
A Patients (7)	Controls (7)	3,712±2,448*
B Controls (7)	Patients (7)	6,226±5,637*
C Controls (7)	Controls (7)	14,326±3,968

* P value A vs. C = <0.01. B vs. C = <0.025. A vs. B = NS.

were performed in pooled AB serum and not in human autologous serum. However, the possibility of such an inhibiting factor cannot be completely ruled out. To distinguish between the responder or stimulator cell abnormality responsible for deficient AMLR in KS, studies with identical or HLA-matched siblings are required. In the stimulator cells, the defect could be with the expression HLA-DR antigens or relative deficiency of B cells, null cells, or monocytes each of which have been claimed to be the major stimulator in AMLR (review in 17, 18). In allogeneic MLR, patient T cells responded poorly and non-T cells from KS were poor stimulators against control responder T cells. Because distinct serologic determinants stimulate (19) and distinct subsets of T cells respond in AMLR and allogeneic AMLR (12, 14), the cellular basis of deficient AMLR in KS cannot be determined without the study of matched siblings.

A number of immunological abnormalities have been reported in homosexuals (3–4). These patients have strong association with CMV infection (6). In our patients, titers of anti-CMV antibodies were also elevated. Carney et al. (20) have reported imbalance of T cell subsets in patients with CMV infection. More recently, Wallace et al. (21) have reported a decrease in T4/T8 ratios in homosexuals who were healthy, or with lymphadenopathy or with KS, though the ratios were most markedly decreased in the latter group. Natural killer (NK) function has been suggested to play an important role in immune surveillance against tumor and certain viruses (22). Tomonari (23) has reported generation of NK in AMLR. Therefore, deficient AMLR in KS could be associated with decreased NK that might play a role in the increased susceptibility to viral infection and development of KS in homosexuals. The role of AMLR in immune surveillance and resistance to viruses is further supported by its deficiency in Hodgkin's disease (24, 25), colon carcinoma (25), and infectious mononucleosis (26).

In the allogeneic MLR, cytotoxic T cells are generated. In the animal model, Zinkernagel and Doherty (27) demonstrated that cytotoxic T lymphocytes (CTL) recognize foreign antigens such as viruses, and histocompatibility antigens in conjunction with products of the major histocompatibility complex (MHC). In addition MHC genes have been shown to function as regulatory immune response genes by controlling the magnitude of CTL response to several foreign antigens in conjunction with particular H-2K and H-2D specificities. The human MHC (HLA) have been demonstrated to play two roles in the generation and expression of CTL responses to virus infected cells (reviewed in 28). (*a*) CTL can only recognize viral antigens in conjunction with antigens, encoded by HLA-A and B genes. Rickinson et al. (29) demonstrated that CTL reactivated in vitro do indeed recognize an Epstein-Barr virus-associated antigen in conjunction with HLA-A and B region gene products on the surface of Epstein-Barr virus-infected B cells. (*b*) HLA-linked genes may control the capacity to generate T cell responses to a given virus or to virus in conjunction with particular self HLA-A and B antigens. Biddison et al. (30) have suggested the role of helper T cells in the stimulation of antiviral CTL precursors. It is also proposed that HLA-DR genes could control the magnitude of a CTL responses by control of helper T cell stimulation. In the allogeneic MLR both OKT4 (helper/inducer phenotype) and OKT8$^+$ (suppressor/cytotoxic phenotype) T cells respond (13). Taken together, a defect in the responder T as well as of stimulator non-T cells in MLR in patients with KS, could result in lower magnitude of CTL and failure of CTL to respond to self HLA antigen plus virus that could explain the susceptibility to viruses in this group of individuals.

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