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## **PNEUMOCYSTIS CARINII PNEUMONIA AND MUCOSAL CANDIDIASIS IN PREVIOUSLY HEALTHY HOMOSEXUAL MEN**

### **Evidence of a New Acquired Cellular Immunodeficiency**

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**Abstract** Four previously healthy homosexual men contracted *Pneumocystis carinii* pneumonia, extensive mucosal candidiasis, and multiple viral infections. In three of the patients these infections followed prolonged fevers of unknown origin. In all four cytomegalovirus was recovered from secretions. Kaposi's sarcoma developed in one patient eight months after he presented with esophageal candidiasis. All patients were anergic and lymphopenic; they had no lymphocyte proliferative responses to soluble antigens, and their responses to phytohemagglutinin were markedly reduced. Monoclonal-antibody analy-

sis of peripheral-blood T-cell subpopulations revealed virtual elimination of the Leu-3+ helper/inducer subset, an increased percentage of the Leu-2+ suppressor/cytotoxic subset, and an increased percentage of cells bearing the thymocyte-associated antigen T10. The inversion of the T helper to suppressor/cytotoxic ratio suggested that cytomegalovirus infection was an important factor in the pathogenesis of the immunodeficient state. A high level of exposure of male homosexuals to cytomegalovirus-infected secretions may account for the occurrence of this immune deficiency. (N Engl J Med. 1981; 305:1425-31.)

**A**QUIRED T-cell defects are well known to occur in adults with untreated Hodgkin's disease, sarcoidosis, and viral infections.<sup>1,2</sup> These non-iatrogenic T-cell deficiencies are marked by cutaneous anergy and diminished proliferative responses to mitogens and antigens in vitro. Opportunistic infections rarely occur in the absence of immunosuppressive therapy. We recently treated several young, previously healthy, homosexual men for multiple episodes of *Pneumocystis carinii* pneumonia, extensive mucosal candidiasis, and severe viral infections.<sup>3</sup> The clinical manifestations and studies of cellular immune function in these patients indicated a similar severe acquired T-cell defect. Several lines of evidence suggested that cytomegalovirus infection was a major factor in the pathogenesis of the immunocompromised state. This syndrome represents a potentially transmissible immune deficiency.

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### **METHODS**

#### **Antibody Assays**

Antibodies to cytomegalovirus, adenovirus, and herpes simplex were determined by complement fixation. Antibody to cytomegalovirus was measured in Patient 2 by the anticomplement immunofluorescence method. Hepatitis B surface antigen and antibodies to hepatitis B surface and core antigens were determined by radioimmunoassay. Antibodies to Epstein-Barr virus were measured in the laboratory of Dr. Werner Henle.

#### **Lymphocyte Separation and Identification**

Peripheral blood was collected in heparin, and lymphocytes were separated on a Ficoll-Hypaque gradient as previously described.<sup>4</sup> T lymphocytes were identified by their ability to form spontaneous rosettes with sheep erythrocytes (E rosettes).<sup>5</sup> B lymphocytes were identified by staining immunoglobulin-bearing cells with a fluorescein-conjugated polyvalent goat antihuman immunoglobulin antiserum.<sup>6,7</sup>

#### **Stimulation of Lymphocytes by Phytohemagglutinin and Antigens**

Blastogenic stimulation of peripheral-blood lymphocytes by phytohemagglutinin (Burroughs-Wellcome) was tested as previously described.<sup>5</sup> The lymphocyte response to candida antigen (Hollister-Stier Laboratories, Spokane, Wash.) and streptokinase-streptodornase (Varidase, Lederle Laboratories) was assayed by mixing antigens with  $2 \times 10^5$  Ficoll-Hypaque-purified peripheral-blood lymphocytes in 0.2 ml of RPMI 1640 supplemented with L-glutamine, streptomycin, penicillin, and 10 per cent heat-inactivated human AB serum in round-bottom microtiter plates. The candida antigen was used at a final concentration of 1:20, and the Varidase

antigen at 20 units of streptokinase and 5 units of streptodornase per 0.2 ml. The cells were cultured for six days and pulsed with [<sup>3</sup>H]thymidine for 16 hours. The phytohemagglutinin and antigens were titrated to obtain maximum stimulation.

### Viral Identification

Viral cultures were performed in human-embryonic-lung fibroblasts (WI38), human-foreskin fibroblasts (Flow 7000), and primary cultures of African-green-monkey-kidney cells. The cultures were maintained at 36°C for five weeks. All isolates were presumptively identified by characteristic cytopathic effect and subsequently confirmed by neutralization<sup>4</sup> or immunofluorescence staining<sup>5</sup> with specific antisera.

### Monoclonal Antibodies

Leu-1, Leu-2, and Leu-3 antibodies were generously provided by Becton-Dickinson (Mountain View, Calif.). Leu-1 recognizes the same surface antigen that monoclonal antibodies OKT-1 and T101 recognize; this antigen is present on mature circulating T cells.<sup>10-12</sup> Leu-2 recognizes the same surface antigen as OKT-8; this antigen is reportedly present on T cells that mediate cytotoxic and immunosuppressive events.<sup>11,13,14</sup> Leu-3a recognizes the same surface antigen as OKT-4, and this antigen is found on T cells showing helper and inducer activity.<sup>13-15</sup> Previous studies in our laboratory have indicated that Leu-2 and OKT-8 define very similar or identical T-cell subsets. Likewise, Leu-3 and OKT-4 recognize equivalent subpopulations. T101 is present on 95 per cent of thymocytes and on less than 5 per cent of peripheral-blood T cells.<sup>16</sup> All monoclonal antibodies were stored at 4°C before their use. Purified mouse myeloma protein of the IgG2a class (RPC 5, Bionetics, Kensington, Md.) served as a negative control for immunofluorescence assays.

### Direct Immunofluorescence Assays

Cells were washed twice before analysis;  $0.5 \times 10^6$  cells were used for each assay. After incubation for 45 minutes at 4°C in 100  $\mu$ l of medium with 2 per cent heat-inactivated neonatal-calf serum and 5  $\mu$ l of monoclonal-antibody solution, cells were washed twice by centrifugation and resuspended in 100  $\mu$ l of medium containing 3  $\mu$ g of fluorescein-conjugated goat antimouse IgG (TAGO, Burlingame, Calif.). After another 45-minute incubation at 4°C, the cells were washed twice in medium containing 0.2 per cent sodium azide and resuspended in one drop of 30 per cent glycerol in phosphate-buffered saline (0.1 M phosphate, pH 7.4) plus 0.2 per cent sodium azide. The cells were examined on a fluorescence microscope equipped with epi-illumination. A minimum of 200 cells were examined for surface immunofluorescence.

### PATIENTS

Tests of cellular immune function were conducted in the Clinical Research Laboratory of the UCLA School of Medicine.

#### Patient 1

A 33-year-old homosexual man was transferred to the UCLA Medical Center on March 16, 1981, for evaluation of recurrent fever and mucocutaneous candidiasis. He had been well until October 1980, when he was admitted to another hospital because of fever and cervical lymphadenopathy. The white-cell count was 6600, with 65 per cent neutrophils, 6 per cent band forms, 23 per cent lymphocytes, and 6 per cent monocytes. Atypical lymphocytes were absent. Test results for latex fixation, antinuclear antibody, venereal disease reaction level (VDRL), Monospot, toxoplasmosis titer, and febrile agglutinins were negative. The antibody profile to Epstein-Barr virus indicated remote infection. The serum contained antibodies to hepatitis B surface and core antigens, but hepatitis B surface antigenemia was absent. The serum aspartate aminotransferase was 388 IU per liter ( $3.12 \mu\text{mol} \cdot \text{sec}^{-1}$  per liter).

The patient received prednisone for two weeks, during which the fever resolved. In December 1980 fever (temperatures to 39°C) recurred, and a two-month course of daily prednisone was administered. Severe oral and perianal candidiasis developed, and the drug dose was rapidly tapered and discontinued. Because of continued fever (39 to 40°C), the patient was transferred to UCLA. Findings included generalized alopecia, moist superficial gluteal erosions, oral thrush, and fungating lesions of the distal index fingers. Rash and lymphadenopathy were absent. Tests of liver enzymes, chest x-ray films, and an upper-gastrointestinal-tract series gave normal results. Cytomegalovirus was recovered from the urine, but not from the blood buffy coat. Herpes simplex and *Candida albicans* were cultured from the pharynx, perianal area, and both fingertip lesions. Serum levels of IgG and IgM were normal, but IgA was elevated to 961 mg per deciliter (normal, 121 to 563 mg per deciliter). Circulating immune complexes, determined by the polyethylene glycol method, were 730  $\mu$ g per milliliter (normal, <310  $\mu$ g per milliliter). The patient was anergic to a battery of five recall antigens, including candida.

Ketoconazole (200 mg twice daily) produced marked improvement of the gluteal and fingertip lesions; however, daily episodes of fever continued. After a five-day course of intravenous acyclovir (9-[2-hydroxyethoxymethyl] guanine) (250 mg per square meter of body-surface area, given three times daily) herpes simplex was no longer recovered from the previously infected sites; however, cytomegalovirus was still cultured from the urine. The patient was discharged but was readmitted one month later with a three-day history of dyspnea, dry cough, and fever. The fingers and perianal lesions were nearly healed. A chest film showed diffuse interstitial infiltrates. Silver stains of bronchial brushings contained numerous *P. carinii* organisms, and smears and cultures for bacteria, acid-fast bacilli, and fungi were negative. Herpes simplex was recovered from bronchial washings. The percentages of both T and B lymphocytes in the peripheral blood were depressed (Table 1). Despite intravenous trimethoprim-sulfamethoxazole (TMP-SMZ), the x-ray findings and arterial oxygenation worsened. A 10-day course of acyclovir (500 mg per square meter, three times daily) was administered. Bronchial brushings and a biopsy specimen obtained on April 24, 1981, were negative for *P. carinii*, acid-fast bacilli, fungi, and *Legionella pneumophila*. Cytomegalovirus was cultured from both specimens. Because of clinical deterioration, TMP-SMZ was stopped and pentamidine was given. An open-lung biopsy performed on May 1 revealed *P. carinii* and morphologic evidence of CMV infection. The patient died two days later. Autopsy findings included extensive *P. carinii* and culture-proved cytomegalovirus pneumonia, with histologic evidence of cytomegalovirus retinitis.

#### Patient 2

A 30-year-old homosexual man was referred to UCLA Medical Center in March 1981 with a four-month history of daily fever and malaise. He had been hospitalized elsewhere in February 1981, at which time generalized lymphadenopathy without hepatosplenomegaly was present. The white-cell count was 4200, with 77 per cent neutrophils, 10 per cent lymphocytes, 8 per cent monocytes, and 5 per cent eosinophils. Atypical lymphocytes were absent. The sedimentation rate was 126 mm per hour. The level of IgG was 2647 mg per deciliter; IgM, 448 mg per deciliter; and IgA, 721 mg per deciliter (the normal upper limits are 1700, 400, and 280 mg, respectively). Circulating immune complexes were elevated to 1530  $\mu$ g per milliliter. Delayed skin tests to five recall antigens were negative. The serum aspartate aminotransferase was 113 IU per liter ( $0.91 \mu\text{mol} \cdot \text{sec}^{-1}$  per liter). A liver biopsy showed mild focal nonspecific hepatitis. Inclusion bodies were absent. The level of antibody to cytomegalovirus, determined by the anticomplement immunofluorescence method, rose from <1:16 to >1:128. Cytomegalovirus was cultured from samples of urine and buffy coat. Antibody evidence of past infection with Epstein-Barr and hepatitis B viruses was detected.

On April 21, 1981, the patient was admitted to UCLA Medical Center, after four days of nonproductive cough, dyspnea, rising fever, and chills. He reported the recent onset of rectal and oral

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thrush. He appeared acutely ill, with a temperature of 39°C. The arterial partial pressure of oxygen during breathing of ambient air was 44 mm Hg, and a chest film showed a diffuse interstitial pattern. Silver staining of bronchial brushings revealed numerous *P. carinii* organisms. A 14-day course of intravenous TMP-SMZ led to resolution of the pneumonia. The white-cell count declined to 1900, with 10 per cent lymphocytes; the platelet count was 80,000. A bone-marrow aspirate and a biopsy specimen were normocellular, with slightly increased numbers of plasma cells. A urine culture grew adenovirus. On June 24, 1981, funduscopic examination revealed multiple discrete white retinal opacities that were consistent with the occurrence of microinfarction of the nerve-fiber layer of the retina. There was no history of visual disturbance. On serial follow-up examinations the lesions had not progressed. The patient was readmitted on August 15, 1981 with gradually increasing dyspnea, cough, and interstitial infiltrates. Bronchial brushings were negative for *P. carinii*; however, numerous pneumocysts were found on open-lung biopsy. Cytomegalovirus was cultured from the tissue specimen. The patient's condition deteriorated despite five days of pentamidine therapy. The fever and pulmonary infiltrates cleared only after a four-week course of high-dose TMP-SMZ, and low-dose prophylaxis was instituted after recovery.

### Patient 3

A 30-year-old, previously healthy, homosexual man was admitted to the UCLA Medical Center with a one-month history of pain on swallowing, oral thrush, leukopenia, and a weight loss of 12 kg. Endoscopy performed after admission revealed distal esophageal erosions and two gastric ulcers. A biopsy specimen of the esophageal mucosa contained intranuclear inclusions but no yeast forms. Virus was not recovered from the initial biopsy sample. The white-cell count was 2300, with 34 per cent neutrophils, 19 per cent band forms, 30 per cent lymphocytes, and 16 per cent monocytes. Atypical lymphocytes were present.

Bone-marrow aspiration and biopsy revealed a slight reduction in the ratio of myeloid to erythroid cells. Serum IgG and IgM were normal, but IgA was elevated to 1061 mg per deciliter (normal, 121 to 363). Circulating immune complexes were elevated at 785 µg per milliliter.

Treatment with amphotericin B reduced the odynophagia. The patient was discharged on February 17, 1981, but was readmitted five days later with fever (temperatures reaching 40°C), dyspnea, and dry cough. The arterial partial pressure of oxygen (room air) was 57 mm Hg, and a chest film showed bilateral interstitial infiltrates. Silver stain of a transbronchial biopsy specimen revealed abundant *P. carinii*. Viral inclusion bodies were absent on tissue sections. An adenovirus was recovered from the bronchial washings and from the stool on two occasions. Treatment with intravenous TMP-SMZ led to prompt resolution of the pneumonia. The percentage of peripheral E-rosette-forming cells was by then markedly decreased (Table 1). The patient was discharged on March 11,

1981, but was readmitted on April 22 because he had severe difficulty in swallowing. Endoscopy revealed confluent plaques of candida and erosions. Additional doses of amphotericin B were administered, producing improvement clinically and endoscopically. Cytomegalovirus was cultured from an esophageal-biopsy specimen. Ophthalmologic examination on May 20 revealed multiple discrete retinal opacities that were asymptomatic. At follow-up examination on May 26 the lesions had increased in size and number. Atypical candidal chorioretinal lesions could not be excluded, and additional amphotericin B (1500 mg) was administered, with little effect on the lesions. On subsequent evaluations, these lesions were thought to represent microinfarctions of the nerve-fiber layer of the retina.

On June 20, dyspnea developed and bilateral lower-lobe interstitial infiltrates were seen on the chest film. A transbronchial biopsy specimen was positive for pneumocystis, which responded to treatment with pentamidine. Cytomegalovirus was cultured from the urine and the biopsy specimen, but not from buffy coat. After recovery, TMP-SMZ prophylaxis was instituted. The patient was readmitted on September 15, 1981, because of progressive cachexia. A violaceous plaque (3 cm in diameter) with a central nodule, which had not been present on previous examinations, was noted on the left wall of the chest; three similar lesions were located in the esophagus. Biopsies revealed Kaposi's sarcoma.

### Patient 4

A 29-year-old homosexual man was referred to UCLA Medical Center on June 5, 1981, with an eight-month history of high fever, lymphadenopathy, fatigue, and a weight loss of 11 kg. He had been hospitalized elsewhere on four occasions in 1981 for diagnostic studies, which were inconclusive. In November 1980 the white-cell count had been 2900, with 73 per cent neutrophils, 10 per cent band forms, 7 per cent lymphocytes, 9 per cent monocytes, and 1 per cent eosinophils. The level of antibody to cytomegalovirus on complement fixation had risen from 1:64 in November 1980 to 1:256 in February 1981. Two biopsies of lymph nodes showed reactive changes, and biopsy of normal-appearing skin revealed a nonspecific perivascular infiltrate. A whole-body computerized tomogram, an abdominal angiogram, and a lymphangiogram for evaluation of possible neoplasia and vasculitis were negative.

On admission the patient reported a two-month history of dysphagia and a two-week history of exertional dyspnea and a morning cough with clear sputum. He appeared cachectic and was febrile (39.5°C). Oral thrush was present. The white-cell count was 4400, with 88 per cent neutrophils, 6 per cent band forms, 3 per cent monocytes, and 3 per cent lymphocytes. Serum levels of IgG and IgM were normal; IgA was elevated to 536 mg per deciliter (normal upper limit, 366). Circulating immune complexes were elevated to 1570 µg per deciliter. The patient was anergic to a battery of five recall antigens. Cytomegalovirus was recovered from the urine. Complement-fixing antibodies to cytomegalovirus, herpes simplex,

Table 1. Levels of Lymphocyte Surface Markers in Patients with Cellular Immunodeficiency.

MARKER	PATIENT 1		PATIENT 2		PATIENT 3		PATIENT 4	NORMAL RANGE
	SAMPLE 1 (3/18/81)	SAMPLE 2 (4/16/81)	SAMPLE 1 (4/21/81)	SAMPLE 2 (5/4/81)	SAMPLE 1 (1/30/81)	SAMPLE 2 (4/22/81)	SAMPLE 1 (6/5/81)	
White-cell count (cells/mm <sup>3</sup> )	3800	4800	3200	2200	2300	1600	4400	4800-10,800
Peripheral lymphocyte count (cells/mm <sup>3</sup> ) *	342	480	320	484	690	480	132	1440-3040
E-rosette formation								
Percentage	62	39	65	44	67	45	44	59-74
Absolute number (cells/mm <sup>3</sup> )	212	187	208	232	462	216	62	849-2249
Surface immunoglobulin-bearing cells								
Percentage	3	2	†	10	9	14	10	5-10
Absolute number (cells/mm <sup>3</sup> )	10	10	†	48	62	67	13	72-304

\*Calculated by multiplying the total leukocyte count by the percentage of lymphocytes in the differential count.

†Quantity was insufficient to perform test.



and adenovirus were not detected. Antibody evidence of past infection with Epstein-Barr and hepatitis B viruses was present. Endoscopy of the upper gastrointestinal tract revealed white plaques in the lower esophagus, and cultures of biopsy specimens grew *C. albicans*. Amphotericin B was given, for a total dose of 300 mg. In view of the uniform occurrence of *P. carinii* pneumonia in the previous three patients, bronchoscopy was performed despite an essentially negative chest film. Numerous pneumocystis organisms were found on bronchial brushings. Treatment with TMP-SMZ was begun and continued for prophylaxis after hospital discharge. On complete ophthalmologic examination the patient was noted to have multiple discrete white retinal opacities, consistent with microinfarction at the level of the nerve-fiber layer ("cotton-wool spots"). He had no visual symptoms.

After discharge the patient remained chronically ill. He was readmitted two months later for recurrence of fever and severe mucosal candidiasis, which responded to amphotericin B.

### RESULTS

Marked lymphopenia was present in all four patients (Table 1). At the first evaluation Patients 1, 2, and 3 had normal percentages of E-rosette-forming cells. In Patients 1 and 2 E-rosette-forming cells were subsequently markedly depressed at the time of *P. carinii* pneumonia. The depressed percentage of T cells in Patient 3 (Sample 2) and Patient 4 had no obvious clinical correlates. The percentage of B lymphocytes was normal except in Patient 1. The four patients had a diminished proliferative response to phytohemagglutinin (Table 2). In those tested, the response to candida and streptokinase-streptodornase was virtually absent. Cells bearing the surface antigen recognized by monoclonal antibodies OKT4 and Leu-3 make the maximal proliferative response to phytohemagglutinin and nearly all the proliferative response to soluble antigens.<sup>19</sup> Thus the results of the mitogen and antigen stimulation studies suggested that these patients had a deficiency of the Leu-3/OKT4 helper/inducer subset. We therefore analyzed peripheral-blood mononuclear cells from these patients with monoclonal antibodies to T-cell subsets on indirect immunofluorescence.

Table 3 shows that peripheral lymphocytes from all four patients contained reduced percentages and total numbers of T cells (Leu-1). Helper/inducer Leu-3+ cells were virtually absent. The percentage of Leu-2+ cytotoxic/suppressor T cells was uniformly increased

Table 2. Stimulation of Mononuclear Cells by Antigens and Phytohemagglutinin in Normal Subjects and Patients.

GROUP	STIMULATOR			
	MEDIUM ALONE	PHYTOHEMAGGLUTININ	CANDIDA ANTIGEN	STREPTOKINASE-STREPTODORNASE
	counts per minute			
Normal subjects (n = 32 [mean ±S.D.])	1,312 ±565	155,983 ±53,652	74,500 ±38,759	87,408 ±24,365
Patients				
1 (3/18/81)	522	27,000	1,594	1,264
2 (4/21/81)	777	81,186	1,930	1,852
3 (5/8/81)	820	5,000	938	*
4 (6/5/81)	666	6,142	*	*

\*Not determined.

Table 3. Characterization of T-Lymphocyte Subsets.

GROUP	LYMPHOCYTE SUBSET				LEU 3/ LEU 2 RATIO
	LEU 1	LEU 2	LEU 3	T10	
<i>per cent lymphocytes reactive with monoclonal antibodies</i>					
Patients					
1	45	57	0	59	0
2	47	52	0	59	0
3	49	57	10	79	0.18
4	67	47	2	81	0.04
Mean $\pm$ S.D.	52 * $\pm 10.1$	53.3 $\dagger$ $\pm 4.7$	3.0 $\dagger$ $\pm 4.76$	69.5 $\dagger$ $\pm 12.1$	0.05 $\pm 0.08$ *
Normal subjects (n = 16 [mean $\pm$ S.D.] )	71.0 $\pm 10.0$	28.0 $\pm 8.0$	46.0 $\pm 12.0$	15.0 $\pm 6.6$	1.6 $\pm 0.74$

\*Significantly different from value in normal subjects (P<0.003).

†Significantly different from value in normal subjects (P<0.0001).

although the absolute numbers (per cubic millimeter) were below normal. The ratio of Leu-3+/Leu-2+ cells was markedly depressed in all patients, relative to the normal control ratio of 1.6. The percentage of cells bearing T10 was increased in all four patients.

### DISCUSSION

These patients presented a distinct and unusual clinical syndrome. All were exclusively homosexual and had been in excellent health before late 1980. Their medical histories did not suggest preexisting immunodeficiency. All were anergic and had infections in which cell-mediated immunity plays the major part in host defense. *P. carinii* pneumonia, extensive mucosal candida infections, and chronic viral shedding were uniformly present. Cytomegalovirus was recovered from secretions in all four patients. A significant rise in specific antibody (Patients 2 and 4) indicated recent infection or reactivation. The presentation in Patient 3 differed in that protracted fever did not precede the development of esophageal candidiasis and pneumocystis pneumonia. The initial illness in Patients 1, 2, and 4 resembled primary cytomegalovirus-induced mononucleosis in previously healthy adults.<sup>17,18</sup> However, the persistence of fever for more than three months and the occurrence of leukopenia, lymphopenia, and opportunistic infection are not features of the cytomegalovirus-mononucleosis syndrome in the normal host. These patients' clinical courses resemble those of primary cytomegalovirus infections in some renal-transplant recipients in whom iatrogenic immunosuppression undoubtedly enhances the severity of infection.<sup>19,20</sup> Although the prednisone administered early in the course of infection in Patient 1 may have impaired the host response to cytomegalovirus, systemic viral infection persisted despite discontinuation of corticosteroids three months before his death. The chronic viral infections in Patients 2, 3, and 4 were clearly unrelated to the administration of immunosuppressive agents. The ret-

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inal lesions (cotton-wool spots) observed in Patients 2, 3, and 4 did not appear to represent ocular infection, since they frequently resolved spontaneously. These lesions are seen in patients with systemic lupus erythematosus, uncontrolled hypertension, or diabetes mellitus. They are described in association with florid cytomegalovirus-retinitis but not as an isolated finding in systemic cytomegalovirus infections. These lesions represent microinfarctions and could be related to the circulating immune complexes documented in these patients. Analysis of the immune complexes for antigenic content is in progress. The explanation for the elevated IgA levels in these patients remains unclear.

The fact that this illness was first observed in homosexual men is probably not due to coincidence. It suggests that a sexually transmitted infectious agent or exposure to a common environment has a critical role in the pathogenesis of the immunodeficient state. Sexually transmitted infections, including cytomegalovirus, are highly prevalent in the male homosexual community.<sup>21-23</sup> In a recent study, 94 per cent of exclusively homosexual men had serologic evidence of cytomegalovirus infection, as compared with 54 per cent of heterosexual men attending the same venereal-disease clinic.<sup>24</sup> The shedding of virus for prolonged periods in many secretions, including semen, facilitates sexual transmission.<sup>25</sup> The four patients described in this report did not know each other. In-depth interviews did not reveal common contacts or knowledge of sexual partners who had been ill. There were no differences among the patients with respect to history of sexually transmitted disease. Patient 3 was highly sexually active and frequented homosexual bars and bathhouses. Patient 1 had lived with one partner for seven years, and Patients 2 and 4 had several regular partners. All patients had serologic evidence of hepatitis B infection in the distant past; however, none had chronic liver disease or hepatitis B antigenemia. There was no history of exposure to a common prescribed or illicit drug.

An increased predisposition to other opportunistic infections has been suspected in immunocompromised hosts who have had cytomegalovirus infection. Coincident pneumocystis pneumonia and cytomegalic inclusion disease was recognized in premature or otherwise debilitated European infants who died in the 1930s and 1940s with what was then termed "interstitial plasma cell pneumonia."<sup>26</sup> After renal and cardiac transplantation, primary cytomegalovirus infection has been associated with greater mortality and an increased incidence of pulmonary superinfection, including *P. carinii*.<sup>27-29</sup> In normal mice, infection with murine cytomegalovirus produces marked immunosuppression and increased mortality from doses of bacteria or candida that are usually not lethal.<sup>30</sup>

In normal subjects cytomegalovirus-induced mononucleosis is associated with in vitro alterations of cellular immune function, which include depressed lym-

phocyte proliferation and interferon production in response to mitogens and herpesvirus antigens.<sup>31,32</sup> Depression of T-cell numbers and of proliferative responses to the degree observed in our patients (Table 2) has not been reported to occur in cytomegalovirus-induced syndromes in normal persons.

Our patients had a marked imbalance of peripheral-blood T-cell subsets, as defined with monoclonal antibodies. The most striking abnormalities were nearly total elimination of Leu-3+ helper/inducer cells and an abnormally increased percentage of Leu-2+ suppressor/cytotoxic cells. Thus, the normal ratio of T helper cells to T suppressor/cytotoxic cells was markedly inverted. The absolute numbers of cells in both subsets were decreased. A very similar reversal of the normal ratio of helper T cells to suppressor/cytotoxic T cells has recently been reported as a uniform occurrence in uncomplicated cytomegalovirus-induced infectious mononucleosis.<sup>33</sup> Although this abnormality was most pronounced during the acute phase of the illness, it persisted in some patients for at least 10 months. The virtual absence of the Leu-3+ subset was undoubtedly the major contributing factor to the severe immune deficiency observed in our patients. The mechanism of this differential effect on the Leu-3+ subset is still unclear, since cytomegalovirus has not been shown to infect subpopulations of lymphocytes directly.<sup>34</sup> Increased immune suppression due to a relative excess of cells with the suppressor phenotype could compound the deficiency. In addition, suppressor monocytes, which have been demonstrated in the peripheral blood of normal persons with cytomegalovirus mononucleosis,<sup>31</sup> could have contributed to the immune deficiency in our patients. The percentage of cells with the thymocyte-associated surface antigen T10 was markedly elevated in all our patients. High percentages of peripheral-blood lymphocytes bearing T10 have been reported in Epstein-Barr virus-induced mononucleosis and in toxoplasmosis.<sup>35,36</sup> We suspect that the increase in T10+ cells in our patients was due to the documented cytomegalovirus infection, since there was no evidence of toxoplasmosis or reactivation of Epstein-Barr virus.

Patient 3 had Kaposi's sarcoma eight months after he had presented with mucosal candida and pneumocystis pneumonia. A recent report described a large number of cases of Kaposi's sarcoma in young homosexual men, four of whom also had pneumocystis pneumonia.<sup>37</sup> In older adults, this neoplasm is associated with serologic evidence of chronic cytomegalovirus infection.<sup>38,39</sup> We recently studied the peripheral-blood T cells of two young homosexual men who presented with exclusively lymphadenopathic Kaposi's sarcoma with no evidence of infection. The pattern was similar to that observed in the four patients described above, with respect to inversion of the Leu-3+/Leu-2+ ratio (0.46 and 0.6). This finding further suggests a relation between the immunodeficiency syndrome and the recent appearance of Kaposi-

si's sarcoma in the homosexual population. Complete evaluation of our other three patients, including endoscopy of the upper and lower gastrointestinal tracts, disclosed no evidence of Kaposi's sarcoma; however, they remain under observation because of the possibility that it will develop.

The reason for the appearance of these two unusual illnesses in male homosexuals remains unclear. It is known that after infection with cytomegalovirus, very high titers of the virus may be shed in the semen of asymptomatic subjects for more than a year.<sup>23</sup> In a recent study cytomegalovirus viremia was detected in 14 per cent of homosexual men 18 to 29 years old.<sup>24</sup> Since urine samples were cultured only once, the true incidence may be even higher. It is therefore likely that sexually active, young homosexual men are frequently reinfected through exposure to semen and urine of sexual partners. Such reinfection with large inoculums of virus before recovery from the cellular immune dysfunction induced by previous cytomegalovirus infection could conceivably lead to overwhelming chronic infection and immunodeficiency or Kaposi's sarcoma. In mice, induction of cytomegalovirus infection by large inoculums of virus leads to destruction of lymphoid elements and profound depression of immune responses.<sup>40,41</sup> The possibility also exists that these syndromes are related to a particular strain of cytomegalovirus transmitted initially within the male homosexual population. We acknowledge the possibility that cytomegalovirus infection was a result rather than a cause of the T-cell defect, and that some other exposure to an undetected microorganism, drug, or toxin made these patients susceptible to infection with opportunistic organisms, including cytomegalovirus. However, at this time cytomegalovirus is highly suspect, in view of its prevalence among male homosexuals and its previously documented potential for immunosuppression.

To date there has been no indication of spontaneous recovery of cellular immunocompetence in our surviving patients. All have continued to have a severe wasting syndrome despite intensive supportive measures. Pneumocystis pneumonia recurred in two of the three patients who did not receive TMP-SMZ prophylaxis. We therefore believe that long-term TMP-SMZ prophylaxis should be initiated in such patients after the first episode of pneumocystis. At present it is unclear whether antiviral and antifungal agents, intensive nutritional support, or immunostimulators will prove useful in the management of this syndrome.

*Note added in proof:* Since submission of this manuscript we have documented this syndrome in an additional four homosexual patients and in two exclusively heterosexual men. Cytomegalovirus was cultured from the semen of one of the heterosexual cases. The latter cases are under intensive study for environmental exposures in common with homosexual patients. To date, we have not encountered female patients with this syndrome.

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## AN OUTBREAK OF COMMUNITY-ACQUIRED PNEUMOCYSTIS CARINII PNEUMONIA

### Initial Manifestation of Cellular Immune Dysfunction

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**Abstract** Eleven cases of community-acquired *Pneumocystis carinii* pneumonia occurred between 1979 and 1981 and prompted clinical and immunologic evaluation of the patients. Young men who were drug abusers (seven patients), homosexuals (six), or both (two) presented with pneumonia. Immunologic testing revealed that absolute lymphocyte counts, T-cell counts, and lymphocyte proliferation were depressed, and that humoral immunity was intact. Of the 11 patients, one was found to have Kaposi's sarcoma,

and another had angioimmunoblastic lymphadenopathy. Eight patients died. In the remaining three, no diagnosis of an immunosuppressive disease was established, despite persistence of immune defects. These cases of pneumocystosis suggest the importance of cell-mediated immune function in the defense against *P. carinii*. The occurrence of this infection among drug abusers and homosexuals indicates that these groups may be at high risk for this infection. (*N Engl J Med*. 1981; 305:1431-8.)

*PNEUMOCYSTIS CARINII* is a ubiquitous organism that infects human beings by a respiratory route. The organism appears to be relatively avirulent, since it rarely if ever causes disease in immunologically competent persons.<sup>1,2</sup> In North America, almost all cases have occurred in patients who have had diagnoses of primary congenital immunodeficiencies or who have received immunosuppressive chemotherapy for malignant neoplastic disease or organ transplantation.<sup>1,2</sup>

Despite the rarity of *P. carinii* pneumonia in previously healthy persons, we recently recognized 11 cases

of this disease in young men with no previous history to suggest immunologic dysfunction. All 11 men were drug abusers or homosexuals or both. Each was found to have similar immunologic defects indicative of abnormal cell-mediated immunity. The clinical, epidemiologic, and immunologic features of this population are the subject of this report.

### METHODS

#### Selection of Patients

Between July 1979 and April 1981, infectious-disease consultants in the New York metropolitan area became aware of several cases of *P. carinii* pneumonia in adults with no history suggestive of immunologic incompetence. Consultants from several medical institutions, who regularly meet at an intercity infectious-disease conference, were queried about similar cases. Thirteen patients were reported from nine hospitals: 11 patients at seven hospitals were made available for this study. A diagnosis of *P. carinii* pneumonia was accepted only if abundant *P. carinii* organisms (identified by methenamine silver or Gram-Weigert stain) were present in areas of lung tissue, if an inflammatory cellular or exudative response was present, and if no other coexistent organisms were demonstrated by pathological examination, by cultivation (for bacteria, fungi, and

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