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# Evidence for Depressed Humoral Immunity to *Pneumocystis carinii* in Homosexual Males, Commercial Plasma Donors, and Patients with Acquired Immunodeficiency Syndrome

LINDA L. WILLIFORD PIFER,[1]* HARVEY B. NIELL,[2,3] SCOTT B. LANGDON,[1] SANDRA BALTZ,[1] STEVEN T. CLARK,[1] CAROL C. EDWARDS,[1] AND DIANE R. WOODS[1]

*Departments of Pediatrics[1] and Medicine,[2] University of Tennessee, Memphis, Tennessee 38163, and Veterans Administration Medical Center, Memphis, Tennessee 38104[3]*

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**Heterosexual controls were found to have significantly higher titers of immunoglobulin G antibody to *Pneumocystis carinii* than did patients with the acquired immunodeficiency syndrome (AIDS) and *P. carinii* pneumonitis, human immunodeficiency virus (HIV) antibody-positive or -negative homosexual male "gay bar" patrons, and HIV antibody-positive or -negative commercial plasma donors. The T-helper/T-suppressor lymphocyte ratios of HIV antibody-negative homosexual male gay bar patrons were slightly depressed (mean = 1.31 ± 0.54) compared with those of heterosexual controls (mean = 1.79 ± 0.32). In addition to other recognized factors, preexisting humoral as well as cell-mediated immune deficits before infection with HIV may help to explain the prevalence of and morbidity and mortality associated with *P. carinii* pneumonitis in AIDS patients.**

Although it is generally accepted that increased susceptibility to opportunistic infections in patients with the acquired immunodeficiency syndrome (AIDS) resulted from the impairment of cell-mediated immunity, more information is needed to detail changes in humoral resistance to specific etiologic agents commonly associated with AIDS. In the present study, attention was focused on *Pneumocystis carinii*, which is the most common life-threatening opportunistic infection observed to date in AIDS patients (16).

In recent years, 51 to 60% of all AIDS patients were initially diagnosed because they presented with clinical symptoms consistent with *P. carinii* pneumonitis (9, 13). Of the total number of patients with AIDS, 71 to 76% had a homosexual or bisexual orientation (3).

In the present investigation, an attempt was made to profile *P. carinii* immunoglobulin G (IgG) antibody titers in AIDS-*P. carinii* pneumonitis patients, homosexual males patronizing "gay bars," heterosexual controls, and commercial plasma donors (CPDs). The overall objective was to gain insight into the influence of life-style and also of exposure to human immunodeficiency virus (HIV) on humoral immunity to *P. carinii*.

## MATERIALS AND METHODS

**Subjects.** Serum samples and heparinized blood from heterosexual controls were obtained from volunteer laboratory workers and from Life Blood, Inc., a volunteer blood donor center (donors were not compensated) in Memphis, Tenn. Serum samples from AIDS patients with acute *P. carinii* pneumonitis who satisfied the Centers for Disease Control criteria for AIDS were obtained from numerous institutions. They were shipped on dry ice to the *P. carinii* serologic reference laboratory supported by the National Cancer Institute as a member of the AIDS Working Group. The serum specimens were accompanied by a complete clinical profile and medical history. All AIDS *P. carinii* pneumonitis patients had biopsy-documented or clinically diagnosed (or both) *P. carinii* pneumonitis.

Local homosexual volunteers who were recruited from gay bars contributed serum specimens for *P. carinii* antibody testing and for T-helper/T-suppressor cell (OKT4/OKT8) determinations. HIV antibody-positive and -negative serum samples, ascertained as such by enzyme-linked immunosorbent assay (ELISA) and, for positive samples, by Western blotting, were obtained from an inner city commercial plasma bank, whose donors were paid for their plasma, after biweekly plasmapheresis.

**Antibody titrations by ELISA.** Titers of IgG antibody to *P. carinii* were determined by a micro-ELISA technique developed in our laboratory (14, 27; L. Pifer, H. Niell, S. Langdon, C. Neely, D. Woods, C. Edwards, S. Roberts, and G. Newton, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 808, 1984). A 100-μl volume of acid-solubilized *P. carinii* containing 3.4 μg of protein suspended in carbonate buffer (pH 9.6) was bound to flat-bottom flexible polyvinyl microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.) by overnight incubation at 4°C.

*P. carinii* organisms purified from the lungs of immunosuppressed Sprague-Dawley rats (5) were used in the preparation of HCl-solubilized antigen (22). The ELISA plates were then rinsed with phosphate-buffered saline containing Tween 20 (PBST) at a dilution of 1:19 and allowed to dry. Each well was treated with 200 μl of blocking solution containing 10% bovine serum albumin in PBST (Kirkegaard and Perry Laboratories, Inc.). All serum specimens were diluted, dropped into the wells after removal of the blocking solution, and allowed to incubate for 1 h at room temperature. The serum dilutions were removed, and the plates were rinsed with PBST. Peroxidase-tagged goat anti-human IgG (heavy and light chain specific [Kirkegaard and Perry]) was added to each well and allowed to incubate for 1 h at room temperature. This solution was then removed, and the plate was rinsed with PBST. Substrate (50 μl) consisting of ABTS {2,2'-di[(3-ethyl-benzthiazoline)] sulfonate} in cacodylic acid

* Corresponding author.

TABLE 1. ELISA IgG titers to *P. carinii* for heterosexual controls, homosexual males, AIDS-*P. carinii* pneumonitis patients, and HIV antibody-positive and -negative CPDs

| *P. carinii* IgG titer | No. (%) with IgG titer[a] | | | | | |
|---|---|---|---|---|---|---|
| | Heterosexual controls[b] (HIV−; *n* = 23) | Homosexual males[c] | | AIDS-*P. carinii* pneumonitis patients[d] (HIV+; *n* = 45) | CPDs[e] | |
| | | HIV+ (*n* = 10) | HIV− (*n* = 56) | | HIV+ (*n* = 40) | HIV− (*n* = 50) |
| 1:8 | 0 (0) | 0 (0) | 0 (0) | 1 (2) | 1 (2.5) | 0 (0) |
| 1:16 | 0 (0) | 0 (0) | 17 (30) | 3 (7) | 5 (12.5) | 2 (4) |
| 1:32 | 0 (0) | 3 (30) | 14 (25) | 3 (7) | 10 (25) | 3 (6) |
| 1:64 | 2 (9) | 5 (50) | 11 (20) | 2 (4) | 13 (32.5) | 10 (20) |
| 1:128 | 1 (4) | 1 (10) | 13 (23) | 12 (27) | 5 (12.5) | 20 (40) |
| 1:256 | 3 (13) | 0 (0) | 1 (2) | 13 (29) | 4 (10) | 8 (16) |
| 1:512 | 14 (61) | 1 (10) | 0 (0) | 8 (18) | 2 (5) | 3 (6) |
| 1:1,024 | 3 (13) | 0 (0) | 0 (0) | 3 (7) | 0 (0) | 3 (6) |
| 1:2,048 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (2) |

[a] The GMTs were as follows: heterosexual controls, 402; HIV-positive homosexual males, 43; HIV-negative homosexual males, 69; AIDS-*P. carinii* pneumonitis patients, 169; HIV-positive CPDs, 139; HIV-negative CPDs, 58.
[b] University laboratory workers and volunteer blood donors (not paid) from Memphis, Tenn.
[c] Gay bar patrons from Memphis, Tenn.
[d] Sera submitted from various institutions in the United States for *P. carinii* serology.
[e] CPDs (paid) from south central United States.

buffer combined with equal parts of $H_2O_2$ was added. The plates were then permitted to incubate in the dark for 30 min at room temperature and were read at 405 nm on an EIA Reader (Abbott Laboratories, North Chicago, Ill.).

Controls consisted of human sera known to be positive or negative for IgG antibody to *P. carinii*, as determined by indirect immunofluorescence methods developed in our laboratory (22). These determinations were subsequently reconfirmed by ELISA. Serum samples from rabbits boosted against *P. carinii* were also included as additional controls (23).

**Lymphocyte subpopulations.** Mononuclear cells from 10 ml of heparinized blood were separated on Ficoll-Hypaque gradients (specific gravity, 1.078) by centrifugation at 400 × *g* for 30 min with the brake off. The mononuclear cells collected were washed in accordance with a protocol described in the Ortho-Mune OKT4/OKT8 kit insert (Ortho Diagnostics, Inc., Raritan, N.J.). In accordance with this protocol, the lymphocytes were incubated with fluorescein-labeled monoclonal antibody directed against the OKT4 or OKT8 lymphocyte subpopulation for 30 min. The cells were enumerated with a fluorescence microscope equipped with a halogen-tungsten lamp. A minimum of 200 cells was evaluated per count, and two or more cell preparations per blood specimen were examined.

**Statistical methods.** The geometric mean titers (GMTs) of IgG antibody to *P. carinii* were determined by using the log (base 10) of each titer. Log titer variance was tested by one-way analysis of variance and Kruskal-Wallis nonparametric analysis (17). The Student-Newman-Keuls multiple comparison test was used to analyze the significance of any intergroup differences. The normality of the data was analyzed by the Kolmogorov-Smirnov goodness-of-fit test (12). A Pearson product-moment correlation was prepared to determine whether a significant relationship might be found between ELISA titers and OKT4/OKT8 ratios.

## RESULTS

**Geometric mean anti-*P. carinii* IgG titers.** The geometric mean anti-*P. carinii* IgG titer for heterosexual subjects (GMT = 402) was significantly higher than those for all other groups tested including HIV antibody-positive or -negative male homosexuals and CPDs, as well as AIDS-*P. carinii* pneumonitis patients ($P < 0.001$ in all comparisons) (Tables 1 and 2). The spectrum of observed GMTs to *P. carinii* ranged widely from 402 in heterosexual controls to 143 and 169, respectively, for HIV antibody-positive and -negative homosexual males.

When all groups were compared with the heterosexual controls, the presence of antibodies to HIV did not appear to be the major factor correlating with the *P. carinii* antibody titer. For example, although HIV antibody-positive CPDs had higher *P. carinii* titers than did HIV-negative donors, both groups had significantly lower GMTs to *P. carinii* than did heterosexual controls. Thus, life-style or socioeconomic elements associated with the four major groups investigated appeared to be of more significance in relation to IgG titers to *P. carinii* than did HIV exposure status.

Although HIV antibody-positive CPDs and AIDS-*P. carinii* pneumonitis patients had higher *P. carinii* titers than did their HIV antibody-negative counterparts, members of both of these groups, whether HIV antibody positive or negative, had substantially lower *P. carinii* titers than did the heterosexual controls. All male homosexuals, regardless of HIV antibody status, had lower GMTs than did the heterosexual controls.

Among HIV antibody-positive CPDs and AIDS-*P. carinii* pneumonitis patients, however, HIV antibody positivity was associated with somewhat higher *P. carinii* titers. This was not the case with HIV antibody positivity in homosexual males. In that group, HIV antibody-positive subjects tended to exhibit lower titers to *P. carinii*.

**Helper/suppressor cell ratios.** Lymphocyte subpopulations in heterosexuals ($n = 20$) and HIV antibody-negative male homosexual gay bar patrons ($n = 26$) had significantly different ($P < 0.001$) profiles (Fig. 1). The OKT4/OKT8 ratios for heterosexual controls and homosexual males were $1.79 \pm 0.32$ and $1.31 \pm 0.54$, respectively. The mean values for heterosexual controls reflected 37.6% OKT4 cells and 21.5% OKT8 cells. In contrast, homosexual male controls had 29.3% OKT4 and 23.9% OKT8 subspecies. Whereas T helper cell values were substantially different in the two groups, reflecting a decrease in the total OKT4 subset, relative values for the OKT8 populations were not significantly different.

TABLE 2. GMTs of IgG antibody to *P. carinii* in AIDS-*P. carinii* pneumonitis patients, homosexual males, CPDs, and heterosexual controls

| Index group (*n*) | GMT | Comparison group (*n*) | GMT | Significance of difference in GMT (*P*) |
|---|---|---|---|---|
| Heterosexual controls (23) | 402 | HIV+ homosexual males (10) | 43 | <0.001 |
| | | HIV− homosexual males (56) | 69 | <0.001 |
| | | HIV+ and HIV− homosexual males (66) | 56 | <0.001 |
| | | AIDS-*P. carinii* pneumonitis patients (45) | 169 | <0.001 |
| | | HIV+ CPDs (50) | 139 | <0.001 |
| | | HIV− CPDs (40) | 58 | <0.001 |
| HIV− homosexual males (56) | 69 | HIV− CPDs (40) | 58 | NS[a] |
| HIV+ homosexual males (10) | 43 | HIV+ CPDs (50) | 139 | NS |
| | | HIV− CPDs (40) | 58 | NS |
| HIV+ and HIV− homosexual males (66) | 60 | HIV+ CPDs (50) | 139 | <0.001 |
| | | HIV− CPDs (40) | 58 | NS |
| AIDS-*P. carinii* pneumonitis patients (45) | 169 | HIV− homosexual males (56) | 69 | <0.001 |
| | | HIV+ homosexual males (10) | 43 | <0.001 |
| | | HIV− CPDs (40) | 58 | <0.001 |
| | | HIV+ CPDs (50) | 139 | NS |

[a] NS, Not significant.

**Relationship between *P. carinii* antibody titers and OKT4/OKT8 data.** A possibly significant relationship between *P. carinii* antibody titers and the relative numbers of helper-inducer T4 cells ($r = 0.462$; $P < 0.05$) was found when data derived from heterosexual controls and male homosexuals were compared by using the Pearson product-moment correlation. Neither the relationship between titer and helper/suppressor cell ratio nor that between titer and OKT8 values was significant.

## DISCUSSION

That various elements pertaining to life-style may influence susceptibility to certain infectious diseases was an accepted concept well before the time of Pasteur. The results of this investigation raise a number of questions concerning the mechanisms whereby immunity to *P. carinii* is established and maintained. These findings may also help to suggest why *P. carinii* pneumonitis is so prevalent in individuals with AIDS.

Previous investigations have shown that most individuals (80 to 90%) develop specific IgG antibody to *P. carinii* by 2 to 4 years of age (20, 22). Lymphocyte stimulation studies revealed that 87% of the lymphocytes of healthy adults "recognized" *P. carinii* antigen (10). The organism appears to be ubiquitous among mammalian species (18) and has been found at postmortem in the lung tissue of approximately 3% of adults without clinical *P. carinii* pneumonitis and in 5.6 to 43% of various groups of pediatric patients (25). Additionally, clinical *P. carinii* infection occurs in organ transplant recipients and individuals with malignancies and primary immune defects, as well as in those receiving therapeutic immunosuppression.

If the concept that *P. carinii* may commonly exist in the lungs of mammals as a saprophyte, a commensal, or component of the incidental pulmonary flora is correct, then its association with nearly all conditions of immunologic compromise, including certain chronic disorders, becomes more predictable. Since AIDS involves insults to both cell-mediated and humoral immunity, the emergence of this opportunist, probably from latency, represents an understandable course of events.

Previous studies have demonstrated the importance of the humoral response, as well as the cell-mediated immune response, in maintaining an effective defense against *P. carinii* (26). More recently, Hofmann et al. (11) failed to detect any IgG antibody to *P. carinii* in AIDS patients by an immunofluorescence assay.

That healthy heterosexual controls possessed substantial IgG titers to *P. carinii* was not unexpected. Likewise, the moderately depressed OKT4/OKT8 ratios in the male homosexual population, a phenomenon that has been reported previously (4, 6, 15), was not interpreted to be unusual. However, the finding that AIDS-*P. carinii* pneumonitis patients, CPDs, and particularly homosexual males, had significantly lower anti-*P. carinii* titers was of interest. Significantly lower titers in AIDS-*P. carinii* pneumonitis patients were not anticipated because polyclonal hypergammaglobulinemia has emerged as a prominent feature of AIDS (1, 7) and because antibody titers to both herpes simplex virus and cytomegalovirus have been reported to be elevated in AIDS patients (8). However, the findings in the present study are more consistent with those of Roberts (24), who described depressed antibody titers to *Coccidioides immitis* in patients with AIDS. Also to be considered is the report by Ammann et al. (2), who found that AIDS patients had a deficient primary antibody response to both polysaccharide and protein challenge antigens.

Finally, a recent study of *P. carinii* titers by an immunofluorescence assay (11) supports the findings of the present study that AIDS-*P. carinii* pneumonitis patients are deficient in IgG antibody to *P. carinii*. In that study, immunocompromised homosexuals without evidence of clinical AIDS were also found to exhibit compromised humoral immunity to *P. carinii*. These findings tend to confirm our observation that ostensibly healthy, HIV antibody-negative homosexual male gay bar patrons with slightly decreased T4/T8 ratios also had significantly depressed anti-*P. carinii* IgG titers. The underlying causes may be multifactorial and may be related to sperm-induced allogeneic immunization (19), the use of alkyl nitrites ("poppers") or other abused drugs, or perhaps other life-style-related elements. It should be emphasized that of the 66 homosexual gay bar patrons participating in this

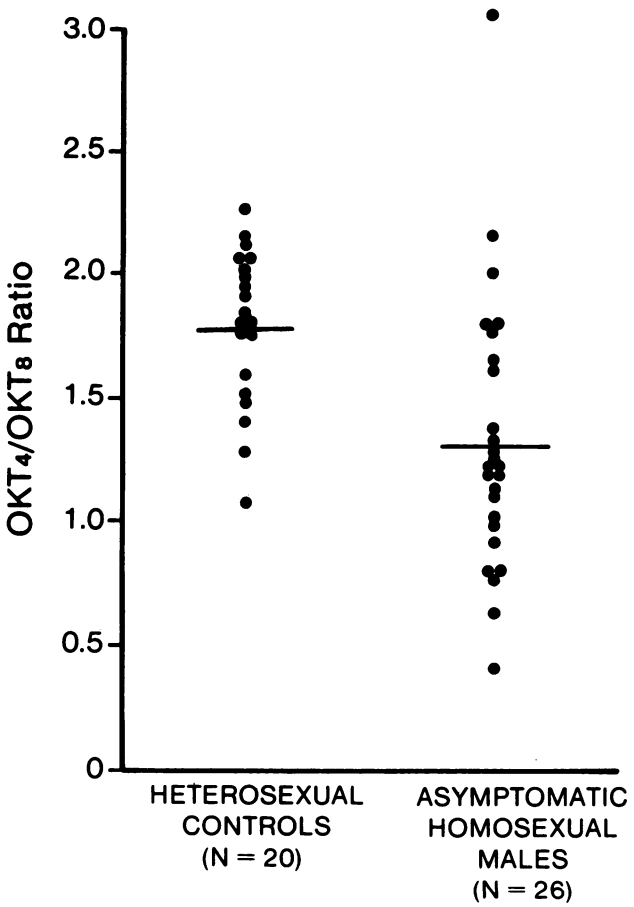


FIG. 1. Lymphocyte subpopulations in heterosexual controls and asymptomatic HIV antibody-negative homosexual male gay bar patrons in an area (Memphis, Tenn.) with a low incidence of AIDS. The horizontal bars indicate mean values.

study, 81% reported routine use of alkyl nitrites and that all subjects reported engaging in sexual activities involving direct anal or oral mucosal contact or both with semen one to seven times weekly (21; L. L. W. Pifer, Y. Wang, T. M. Chiang, R. Ahokas, D. R. Woods, and R. E. Joyner, South. Med. J., in press).

The slightly diminished proportion of T4 cells in homosexual males may help to provide an explanation for the significantly lower titers to *P. carinii*, since the IgG antibody response appears to rely at least in part on the helper-inducer T cells. It should be noted, however, that although the difference between data for homosexual males and heterosexual controls was statistically significant, the observed difference is not viewed as clinically significant. Maintenance of normal immunity to *P. carinii* may depend upon a homeostatic mechanism involving antigen processing, activation of cell-mediated immune functions, and antibody production. Disruption of the T4/T8 cell ratio could readily result in deficits in both cell-mediated and humoral immunity to *P. carinii* pneumonitis. These findings appear to suggest that the deficits may exist in some homosexual males before evidence of infection with HIV.

It should be noted, however, that the T4/T8 cell ratios and the *P. carinii* titers presented in this report were based exclusively on studies of a selected group of homosexual males who frequented gay bars and may not be representative of all men who engage exclusively or primarily in sexual relationships with other males.

The significantly low *P. carinii* titers observed in CPDs are also of interest. The demographic profile of the plasma donors tested reflects an inner city cohort of low socioeconomic status in whom alcohol, drug abuse, poor nutrition, and minimal health care are common. The CPDs tested were allowed to donate plasma as frequently as twice weekly if their total serum protein concentrations and hematocrits met minimum acceptable limits. Since IgG antibody exhibits a half-life of approximately 21 days, it is conceivable that this feature, in addition to the others described, may have contributed to the significantly lower *P. carinii* antibody titers observed in this group. Whether this phenomenon applies to titers of antibody to other infectious agents or is relatively unique to *P. carinii* is unknown and awaits further investigation. This is of more than academic interest since some commercial IgG sources derive their product from paid plasma donors.

In the final analysis, AIDS-*P. carinii* pneumonitis patients, homosexual males, and CPDs appear overall to be similar in their titers of IgG antibody to *P. carinii*. Since AIDS-*P. carinii* pneumonitis patients doubtless respond directly to antigenic challenge consistent with clinical *P. carinii* pneumonitis, this may suggest why their titers were somewhat higher than those of all homosexual males, regardless of HIV antibody status, and HIV antibody-negative CPDs.

Studies of *P. carinii* titers in individuals at risk for contracting HIV infection, in recent HIV seroconverters, and in acute- and convalescent-phase AIDS-*P. carinii* pneumonitis patients, as well as in many other control groups, will be required to obtain an accurate and complete overview of the humoral immunologic profile of *P. carinii*.

In conclusion, our data appear to suggest that homosexual males and individuals who serve as paid plasma donors have depressed humoral immunity to *P. carinii*, independent of their HIV antibody status. In addition to the obvious insults to the immune defenses inflicted by AIDS, this may offer an explanation for the prevalence, morbidity, and mortality of *P. carini* pneumonitis in patients with AIDS.

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