Suppressor Cell Activity among the Peripheral Blood Leukocytes of Selected Homosexual Subjects

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ABSTRACT

Suppressor cell activity was investigated in the peripheral blood leukocytes of 21 homosexual subjects with a presumed prodrome of the opportunistic infection, Kaposi's sarcoma syndrome. Patient lymphocytes, either untreated or irradiated with 4000 rads, were cultured alone or were cocultured with normal lymphocytes. The cultures were stimulated with phytohemagglutinin, concanavalin A, and pokeweed mitogen. The proliferative responses of these cultures were compared to each other and were related to concurrently studied delayed hypersensitivity skin test responses to dermatophyton, candida, varidase, mumps, and purified protein derivative of tuberculoprotein; to the proportion of erythrocyte rosette-forming cells and OKT 3-, OKT 4-, and OKT 8-positive cells; and to the OKT 4:OKT 8 ratio in the peripheral blood leukocytes. Patient blastogenic responses were significantly lower than controls for phytohemagglutinin (29.9 versus 75.3 × 10³ cpm/culture), for concanavalin A (6.9 versus 28.7 × 10³ cpm/culture), and for pokeweed mitogen (7.1 versus 22.4 × 10³ cpm/culture). In 12 of 21 patients, significant suppressor cell activity was detected for all three mitogens. For example, for these 12, the normal controls showed 82.9 × 10³ cpm when cultured alone but only 0.9 × 10³ cpm when cocultured with the patient's cells. Irradiation reversed the suppressor cell activity in nine of 12 of the subjects. Suppressor cell activity was seen with as few as 0.125 × 10⁵ patient lymphocytes added per 10⁶ normal lymphocytes. The percentage of suppression of the normal blastogenic responses correlated significantly with the patient blastogenic responses, the percentage of erythrocyte rosette-forming cells, the percentage of OKT 3- and OKT 4-positive cells, and the helper:suppressor ratio, but not with the percentage of suppressor cells. These data indicate that suppressor cell activity can be found in homosexuals with the prodrome of the opportunistic infection, Kaposi's sarcoma syndrome, and may be responsible for their in vivo immune deficiency. It also suggests that therapeutic maneuvers, which would restore the helper:suppressor ratio towards normal, might benefit the patients clinically.

INTRODUCTION

During the last 2 years, a syndrome of opportunistic infection and Kaposi's sarcoma has been observed, with increasing frequency, among a group of individuals, predominately young male homosexuals (5, 14, 26). These patients manifest a spectrum of abnormal immunological functions during the period when they have severe opportunistic infection, such as Pneumocystis carinii pneumonia. Recently, relatively healthy and ambulatory homosexuals with a normal performance status have also been observed to have immune abnormalities (21). These abnormalities include: skin test anergy; impaired in vitro lymphocyte blastogenic responses to mitogens and antigens; low helper T-cells; an inverted helper:suppressor ratio; elevated serum thymosin α₁ levels; and low total peripheral blood white counts, including mild to moderate anemia, thrombocytopenia, and leukopenia involving all 3 major classes of leukocytes.

One of the most striking and uniform features has been the low level of circulating helper T-cells with normal or slightly elevated suppressor cells resulting in an inverted helper:suppressor ratio (5, 14, 21, 26). While this finding is not unique to this group of patients and has been seen during systemic viral infections (24) in transplant recipients with graft versus host reactions (20) and in some patients with solid tumors, it is a recognized characteristic of these subjects. It would be important to determine whether the abnormal helper:suppressor ratio and the other leukocyte subset abnormalities are simply a cell surface marker phenomenon or whether they are accompanied by increasing suppressor cell activity demonstrable in functional assays. The latter is suggested since some of the patients have poor lymphocyte-proliferative responses (21). To investigate this question, we used a suppressor cell assay first described by us in bone marrow transplant recipients and cancer patients with impaired lymphocyte proliferations in vitro (9). In that report, the patients' lymphocytes were found to suppress the proliferative responses of normal subjects' lymphocytes, and the suppressor cell activity was abrogated by irradiation of the patient cells or by in vitro treatment with the thymic hormones, Thymosin Fraction 5, and thymic humoral factor. In this paper, we report suppressor cell studies on 21 homosexual patients with a high risk of developing the syndrome of opportunistic infection and/or Kaposi's sarcoma, but who were ambulatory with a normal performance status at the time of the study. The prodromal syndrome, which brought these subjects to attention, consists of lymphadenopathy, fever, weight loss, diarrhea, and other nonspecific constitutional symptoms. Approximately one-half of the patients manifested suppressor cell activity in coculture which in most, but not all, individuals could be abrogated by irradiation of the patients' lymphocytes. The results of this assay are correlated with the patients' lymphocyte-proliferative responses, their delayed hypersensitivity skin test reactions, and their leukocyte surface marker analyses.

MATERIALS AND METHODS

Patients. Twenty-one patients were studied. They ranged in age from 26 to 35 years with a median of 31. They were all ambulatory with a
performance status of 0 or 1 (Zubrod scale). They were selected on the basis of having the homosexual life style, multiple sexual partners, multiple episodes of sexually transmitted disease, and one or more documented or suspected episodes related to the presumed prodrome of the syndrome, as noted above. The controls consisted of normal heterosexual subjects ranging in age from 20 to 31 with no known active diseases. Control cells were cultured concurrently with the patients’ cells and were also used in the coculture experiments to detect suppressor cell activity. In addition, 5 pairs of normal heterosexual subjects were studied in coculture to confirm that no suppressor activity was observed in cocultures of normal cells as described previously (9).

Leukocyte Collection and Separation. Peripheral blood was collected by venipuncture and was defibrinated by swirling with glass beads. Mononuclear leukocytes were separated by Ficoll-Hypaque density gradient centrifugation, and leukocyte counts and differentials were performed by light microscopy on wet preparations in hemocytometer chambers and on Wright-Giemsa-stained preparations. Mononuclear cells from the Ficoll-Hypaque interface were washed 3 times in Hank’s balanced salt solution containing 5% fetal calf serum prior to establishing them in culture.

Lymphocyte Cultures. Lymphocyte cultures were done in microtiter test plates, as described previously (13). All points were done in triplicate and, unless otherwise described, contained 1.0 $\times$ 10^5 lymphocytes in 0.2 ml of Roswell Park Memorial Institute Tissue Culture Medium 1640 with 10% heat-inactivated pooled human AB serum. Cocultures contained 1.0 $\times$ 10^5 lymphocytes from each of the 2 allogeneic subjects. The cultures received either no additive or 0.02 ml amounts of PHA$^3$ (Difco Laboratories, Detroit, Mich.), PWM (Grand Island Biological Co., Grand Island, N. Y.), or Con A (Difco). These mitogens were used at reagent concentrations of 1:10 dilution of the stock solution, 1:20 dilution of the stock solution, and 80 $\mu$g/ml, respectively. Cultures were incubated for 3 days and then harvested. Eight hr before harvest, 1 $\mu$Ci of [3H]-thymidine (Amersham/Searle Corp., Arlington Heights, Ill.; specific activity, 1.9 Ci/mmol) was added to each well. The cultures were harvested with a MASH 2 automatic sample harvester, and fiberglass filter discs attached sheep cells were considered positive. T-cell subset surface antigens were quantitated with the OKT 3, OKT 4, and OKT 8 monoclonal antisera (Ortho-mune; Ortho Diagnostics, Raritan, N. J.). Whole-blood specimens (0.1 ml) were lysed with lysing buffer for 10 min, and the leukocytes were washed with 0.9% NaCl solution and incubated for 30 min with 0.01 ml of the appropriate fluorescein isothiocyanate-coupled antibody. The cells were again washed with 0.9% NaCl solution, and fluorescent lymphocytes were counted in an Ortho Spectrum III cell counter (10).

RESULTS

Table 1 shows the in vitro lymphocyte blastogenic responses of the studied subjects and the concurrent normal controls. It can be seen that the study patients had markedly depressed lymphocyte blastogenic responses. In 8 of the subjects, the responses were completely below the normal range, while in 6 of these, proliferative responses to PHA were virtually absent. The differences between the patient and normal responses were statistically significant. Proliferation in unstimulated cultures was statistically significant. Proliferation in stimulated cultures was also examined. It did not differ between patients (748 $\pm$ 282 cpm) and normals (751 $\pm$ 253 cpm).

Table 2 shows the suppressor cell data. In general, only individuals who themselves had impaired lymphocyte-proliferative responses manifested suppressor cell activity. Therefore, only their data are shown. Twelve of the 21 patients manifested suppression. Marked suppression of lymphocyte-proliferative responses was seen most often in PHA- and Con A-stimulated cultures, while PWM cultures less frequently showed suppression. Only 4 of the subjects manifested suppressor cell activity against all 3 mitogens. In 9 of 12 of the individuals, the suppressor cell activity was partially or completely reversed by irradiation variance of replicate cultures (8). In order to be defined as showing suppressor activity, the cpm of the coculture of normal and patient cells was arbitrarily required to be less than the cpm of both normal cells and normal cells cultured at double the usual concentration.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>PHA</th>
<th>Con A</th>
<th>PWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>29.9 ± 32.0$^a$</td>
<td>6.9 ± 8.3</td>
<td>7.1 ± 6.4</td>
</tr>
<tr>
<td>Controls</td>
<td>75.3 ± 41.0</td>
<td>28.7 ± 29.7</td>
<td>22.4 ± 20.7</td>
</tr>
<tr>
<td>$p$ (t test)</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

$^a$ Mean ± S.D.

3The abbreviations used are: PHA, phytohemagglutinin; PWM, pokeweed mitogen; Con A, concanavalin A.

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of the patient cells. In the remaining 3 subjects, reversal of suppressor cell activity by irradiation did not occur. In coculture experiments in 5 pairs of allogeneic normal individuals, set up using the same system, there was no suppressor cell activity noted (data not shown).

Since suppressor cell activity did not persist in 9 of 12 instances after irradiation of patient cells, there was a highly significant correlation between the patient lymphocyte responses to mitogens and the response of normal plus patient in coculture before but not after irradiation (Table 3). In contrast, normal lymphocyte responses correlated highly with responses of normal plus irradiated patient cocultures, showing the restoration of the normal lymphocyte responses in coculture when the patient cells were irradiated. There was no significant correlation between the normal and patient responses in separate cultures, as expected. The correlation between the blastogenic responses of the patient cells and the normal plus patient cocultures and the lack of correlation between patient and normal cultures are shown graphically in Chart 1.

In addition to the above, Tables 2 and 3 also show data on cultures containing 1 x 10⁵ and 2 x 10⁵ normal cells. There was no significant inhibition or augmentation of the normal responses by doubling the number of cells (as a control for the potential effects of crowding).

In 7 cases, including 5 showing suppressor cell activity, we cultured patient cells with irradiated normal cells. The proliferation of patient cells was usually boosted slightly by the irradiated normal cells, adding further support to the concept of the unidirectional nature of the suppressor cell activity (Table 4). Chart 2 shows the dose-response data in regard to the numbers of patient cells required to suppress the proliferative responses of a fixed number of normal subject cells. It can be seen that some degree of suppressor cell activity was manifest with

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>Normal</th>
<th>2 times normal</th>
<th>Patient</th>
<th>Normal + patient</th>
<th>Normal + patient irradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td>82.9</td>
<td>58.3</td>
<td>3.8</td>
<td>0.9</td>
<td>63.3</td>
</tr>
<tr>
<td>Con A</td>
<td>38.7</td>
<td>40.5</td>
<td>2.7</td>
<td>20.8</td>
<td>39.5</td>
</tr>
<tr>
<td>PWM</td>
<td>46.5</td>
<td>63.5</td>
<td>3.8</td>
<td>21.5</td>
<td>44.0</td>
</tr>
</tbody>
</table>

Table 2
Evidence for suppressor cell activity in cocultures of patient and control subject mononuclear cells

Data shown are for the 12 patients who showed suppressor cell activity and their concurrent controls. Cultures contained 1 x 10⁵ or 2 x 10⁵ lymphocytes. p values by Wilcoxon test: normal versus 2 times normal, not significant; normal versus normal plus patient, p < 0.001; normal versus normal plus patient (irradiated), not significant; normal plus patient versus normal plus patient (irradiated), p < 0.01.

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>Cells in culture (cpm x 10⁵/culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
</tr>
<tr>
<td>PHA</td>
<td>41.3 ± 4.4</td>
</tr>
<tr>
<td>Con A</td>
<td>8.4 ± 8.9</td>
</tr>
<tr>
<td>PWM</td>
<td>11.2 ± 7.7</td>
</tr>
</tbody>
</table>

Table 4
Effect of irradiated normal cells on the proliferation of 7 patients' lymphocytes to mitogens

as few as 0.125 x 10⁵ patient cells added to 1.0 x 10⁵ normal cells.

Table 5 gives the data on blastogenic responses, proportion of T-cells and T-cell subsets in the peripheral blood of these study subjects, and their in vivo delayed hypersensitive responses (number of positive tests of 5) in relationship to the percentage of suppressor cell activity. The greater impairment of a number of the parameters in relationship to the presence of suppressor cell activity is evident. However, this was statistically significant only for the PHA response.

Table 6 gives the correlation coefficients between the percentage of suppression in the cocultures and proportions of total T-cells, helper cells, suppressor cells, the helper:suppressor ratios, patient blastogenic responses, and the delayed hypersensitivity responses of the individuals. There were significant inverse correlations between the suppressor cell activity (percentage of suppression) and the PHA, Con A, and PWM responses of the patient cells; between the percentage of suppression of PWM and the total T-cells measured by erythrocyte-forming rosettes, and between the percentage of suppression of Con A and PWM versus the percentage of total T-cells measured by OKT 3, the percentage of helper cells and the helper:suppressor ratios. There was no correlation between the percentage of suppression in the cocultures and the delayed hypersensitivity responses or the percentage of suppressor cells.

Eleven of the patients were evaluated for suppressor cell activity for immunoglobulin synthesis (12) in response to in vitro stimulation with Epstein-Barr virus (T-cell independent) and PWM (T-cell dependent). Only one of the patients and one of 5 concurrently studied controls showed suppression of immunoglobulin synthesis (data not shown). Therefore, we conclude that this


**Table 5**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of positive skin tests</th>
<th>Mitogens (cpm x 10^5/culture)</th>
<th>% of positive lymphocytes</th>
<th>OKT 4:OKT 8 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>PHA</td>
<td>Con A</td>
<td>PWM</td>
</tr>
<tr>
<td>Suppressor cell activity</td>
<td>Present (n = 12)</td>
<td>1.2 ± 1.3^b</td>
<td>17.5 ± 24.5</td>
<td>4.7 ± 4.7</td>
</tr>
<tr>
<td>Absent (n = 9)</td>
<td>1.6 ± 1.2</td>
<td>46.4 ± 34.8</td>
<td>11.6 ± 11.4</td>
<td>8.9 ± 9.4</td>
</tr>
<tr>
<td>p value^c</td>
<td>0.49</td>
<td>0.03</td>
<td>0.30</td>
<td>0.64</td>
</tr>
</tbody>
</table>

^a E= OKT 3, OKT 4, and OKT 8, monoclonal antiserum.
^b Mean ± S.D.
^c Comparison of parameters in those with and without suppressor cell activity (t test).

**Chart 2.** Cocultures of constant numbers of one cell (N) with increasing numbers of autologous cells (N+N), irradiated patient cells (N+PX), or patient cells (N+P) are shown. Profound suppression of normal cell responses (70,000 cpm) by patient cells (20,000 cpm) and moderate suppression by irradiated patient cells (38,000 cpm) are shown. The lowest number of cells tested showing suppressive activity was 0.125 x 10^9. Bars, S.E. for a single patient study.

**DISCUSSION**

During the last 2 years, a syndrome of opportunistic infection and Kaposi's sarcoma has been noted with increasing frequency, mainly, but not exclusively, among young homosexual men (5, 14, 21, 26). This syndrome has apparently spread outwardly from New York, N. Y. (14), next involving patients on the West Coast (5), then patients in other American cities (21), and most recently patients in England (15), France (23), and Spain (29). The immunological syndrome includes impaired in vivo delayed hypersensitivity, deficient in vitro lymphocyte-proliferative responses, and markedly reduced levels of helper cells, the latter resulting in an inverted helper:suppressor ratio. Hematological abnormalities and deficient monocyte number and functions have also been observed. The data indicate an impairment of those host defense mechanisms involved in resistance to viral, fungal, and acid fast bacterial infection. It is not surprising, therefore, that these patients apparently suffer from a variety of severe and even life-threatening infections, including disseminated cytomegalovirus disease, P. carinii pneumonia, disseminated tuberculosis, atypical acid fast bacterial infection, disseminated candidiasis, a variety of bacterial infections, and infections with enteric pathogens.

Even more disturbing has been the incidence of cancer, mainly Kaposi's sarcoma, but also p.o. (1) and rectal (2) squamous cell carcinoma and lymphoma (7). The etiology of the syndrome is unknown, but it has been attributed to the immunosuppressive effects of viral infection, due particularly to cytomegalovirus (22) but also perhaps hepatitis or Epstein-Barr virus; to the immunosuppressive effects of drug abuse (4), particularly marijuana and the nitrites, and to other factors, such as the use of corticosteroid creams in the treatment of various skin eruptions (18, 19). Another possible factor is the immunosuppressive effects of semen (11). Of greatest importance is that the severe immunodeficiency has now been noted not only in patients with opportunistic infection and Kaposi's sarcoma, but also in relatively healthy ambulatory patients (21). Thus, the immune deficiency clearly precedes the development of the syndrome.

This report is the first description of which we are aware of documented functional suppressor cell activity in this group of patients. Increased suppressor cell activity was anticipated because of the inverted helper:suppressor ratio which appears to be characteristic of the full-fledged syndrome and its prodrome. Suppressor cell activity could certainly be related to virus infection in that both cytomegalovirus infection (22) and Epstein-Barr virus infection (3) are associated with a selective proliferation of suppressor cells and a selective loss of helper cells. All of the patients had had cytomegalovirus infection as manifested by elevated antibody titers to that virus. Not only is the syndrome

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^The authors wish to thank Dr. C. Lawrence of Baylor Medical College for the conduct of these experiments.
manifested by an increased proportion of suppressor cells, but more important, it is manifested by active suppression of lymphocyte-proliferative responses. Several findings are of interest in this regard. (a) These patients apparently did not manifest suppression of in vitro antibody synthesis. (b) The percentage of suppression did not correlate inversely with the percentage of suppressor cells but did with the T-cells, helper cells, and helper:suppressor ratio. Therefore, it is the relative proportions of helper to suppressor cells and not the absolute numbers of the latter which appear to determine functional suppressor activity.

The relationship of these findings to the development or manifestation of either infection or cancer in these patients is not known at present. However, it may become apparent through long-term follow-up of the patients. The data are also of interest in regard to the known role of suppressor cell activity in the occurrence and progression of malignant disease. Thus, several studies in animal tumor models indicate that the administration of suppressor cells can accelerate the rate of tumor growth (28) and that the administration of treatment directed against suppressor cells may have antitumor therapeutic activity. For example, the administration of antisuppressor cell antibody (6) or the administration of drugs such as cimetidine (27), which can abrogate T-suppressor cell function, and indomethacin (16), which can abrogate suppressor macrophage function, may have antitumor effects in animal tumor systems. This is also of interest since clinical studies using these agents to abrogate suppressor cell activity in vitro and in vivo are now under way in cancer patients.

In regard to the population of patients with opportunistic infection and/or Kaposi’s sarcoma, it will be of interest to correlate the findings of suppressor cell activity with subsequent disease manifestations, if any. Furthermore, it will be of interest to attempt the evaluation of drugs causing a reduction of suppressor cell activity in vitro in these patients. If suppressor cell activity can be altered, then the manifestations of immunodeficiency and opportunistic infection might be considered as indicators for in vivo antisuppressor cell treatment.

Relative to the points being made in the last 2 paragraphs, we are continuing these studies in normal subjects, healthy monogamous homosexuals, and patients with opportunistic infection and/or Kaposi’s sarcoma. While data are incomplete, it appears that the healthy, monogamous homosexuals do not manifest impaired blastogenesis or suppressor cell activity.

REFERENCES

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