Impaired in Vitro Interferon, Blastogenic, and Natural Killer Cell Responses to Viral Stimulation in Acquired Immune Deficiency Syndrome

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ABSTRACT

The in vitro immune response to herpes simplex virus (HSV), type 1, strain 539, HSV type 2, strain 316D, and cytomegalovirus was studied in 20 patients (14 with acquired immune deficiency syndrome, four with the acquired immune deficiency syndrome-related symptom complex, and two sexually active asymptomatic homosexuals) and 18 heterosexual controls. Peripherally blood mononuclear cells were cultured with $2 \times 10^7$ plaque-forming units of heat-inactivated viruses, their lymphocyte blastogenic responses were measured after 5 days in culture by $[^3H]thymidine incorporation, their interferon production was measured after 24 hr and 5 days, and natural killer (NK) cell activation was measured after 24 hr and 5 days of culture. Blastogenic responses to viruses were significantly low for only HSV, type 1: $1.75 \times 10^3$ cpm in patients’ cells compared to $6.36$ for controls. Interferon responses to all three viruses were significantly low at both 24 hr and 5 days; e.g., HSV, type 1: $139$ IU/ml in patients’ cells compared to 777 for controls at 24 hr. NK cell responses of patients were lower than those of controls when tested fresh and after 24 hr of incubation: 6.1 versus 11.7% and 9.2 versus 16.8% target cell lysis, respectively. Exposure to viruses boosted NK cell responses of both patients and controls’ cells, but boosting was generally greater among the normal rather than the patients’ cells. The abnormalities of response were present in all three patient groups. Addition of interleukin-2 in vitro increased the patient and control blastogenic and NK responses but did not augment the interferon responses. The in vitro responses to both HSV, type 1, and HSV, type 2, correlated significantly with our conventional assays of the percentage and absolute level of T4+-helper lymphocytes in the blood and the blastogenic responses to mitogens, such as phytohemagglutinin, pokeweed mitogen, and concanavalin A. This system should be useful for the study of host defense in acquired immune deficiency syndrome patients and those in high-risk groups, and also for the in vitro evaluation of immunomodulators.

INTRODUCTION

AIDS has become a major epidemic in the United States (1). The overall mortality from the associated Kaposi’s sarcoma and opportunistic infection is 40%. Approximately 3200 cases have been described in the United States in the last 3 years. The immunodeficiency has been well described in these patients. Recently, it has become evident that a much larger number of individuals have a disease entity which may precede the development of AIDS (21). This is a syndrome characterized by fever, night sweats, lymphadenopathy, weight loss, diarrhea, and fatigue with similar immunological findings to those seen in AIDS. This has been referred to as the ARC. In addition, it appears that patients in at least one of the high-risk groups for the development of ARC and AIDS may also have similar immunological abnormalities (17). Some of the abnormalities include impaired delayed hypersensitivity, low relative and absolute levels of helper cells, inverted helper:suppressor ratio, impaired lymphocyte blastogenesis, impaired NK cell activity, elevated serum thymosin-α1 (9), serum β2-microglobulin (26), serum lysozyme (17), hypergammaglobulinemia, and circulating immune complexes (18). One of the prime immunological defects appears to be the low peripheral blood T-helper cells with an inverted ratio of T-helper to T-suppressor cells.

The etiology of the disease is unknown, but a transmissible viral agent superimposed on a preexisting immune deficiency has been hypothesized. A leading candidate at present is human T-cell leukemia virus (2, 4, 5). The fundamental mechanism of the immunodeficiency is also not well understood but presumably relates to a direct immunosuppressive effect of the virus. The consequences, of course, are the development of opportunistic infection and unusual cancers.

Several workers have attempted to investigate the mechanism and have demonstrated recently impaired IL-2 production and response (25), impaired helper cell activity with impaired immunoglobulin synthesis (11), and augmented suppressor cell activity (7). In addition, impaired production of interferon upon in vitro stimulation with herpes simplex viruses has been noted in AIDS patients (14).

Because of the high incidence of opportunistic viral infections in these patients, associated with T-cell immunodeficiency and the above-noted findings, we elected to further study the mechanism by characterizing the in vitro response to 2 viruses which have been associated with AIDS. Peripheral blood leukocytes from patients were exposed in vitro to CMV and HSV, and the lymphocyte-proliferative responses, the generation of NK cells, and the induction of interferon were studied. Also, the effects of IL-2 added in vitro on these parameters were also studied.

MATERIALS AND METHODS

Twenty patients were studied. They were chosen at random from a group of 300 sexually active homosexual men between the ages of 20
and 50 being followed in the Cancer Prevention Clinic at M.D. Anderson
Hospital and Tumor Institute. None was receiving treatment at the
time of study. Fourteen had AIDS; 4 had ARC; and 2 were symptom\free,
had never had AIDS-related symptoms or significant prior medical his\tories. These symptom-free patients were included, since we have
demonstrated that these high-risk patients have the same type of
abnormalities as ARC and AIDS patients. Eighteen heterosexual normal
controls, aged 20 to 50, were also studied. None of the patients was on
therapy nor had any received prior therapy for AIDS. The individual
controls were studied concurrently with one or 2 of the patients studied.

The viral materials were prepared as follows. HSV-1, strain 539, and
HSV-2, strain 316-D, were replicated in rabbit skin cells to a titer of
approximately $2 \times 10^8$ plaque-forming units/ml. Cellular debris was
removed by centrifugation at 2500 x g for 30 min. Supernatant fluid was
heated at 56° for 60 min to inactivate virus. The virus pool was aliquoted
in 1-ml volumes and stored at ~70° until used. Control antigen consisted
of supernatant fluid from rabbit skin cells treated identically to the virus
preparation.

$^3$- CMV and $^5$- CMV control antigen were prepared as follows. A stock
laboratory strain of $^3$- CMV (AD169) was grown to confluence in human
foreskin fibroblast-containing flasks. Cellular debris was removed by
centrifugation at 2500 x g for 30 min. Supernatant fluid was heated at
56° for 30 min to inactivate the virus. Aliquots of the virus pool were
stored at ~75° until used. Control antigen was prepared from uninfected
human foreskin fibroblast monolayers that received the same treatment
as virus-infected cells.

Lymphocyte cultures were set up as follows (8). Peripheral blood was
obtained by venipuncture and defibrinated by swirling with glass beads.
RBC were removed, and peripheral blood mononuclear cells were pre\pared by Ficoll-Hypaque density solution centrifugation. Leukocyte sus\pensions were washed 3 times in HBSS and resuspended in RPMI 1640
medium with 10% fetal bovine serum. Cells were cultured in round-bottomed Microtest-II plates (Falcon). Each culture contained $1.5 \times 10^8$
peripheral blood mononuclear cells and 0.2 ml of complete medium.
Lymphocytes were cultured either unstimulated or stimulated with PHA,
CON-A, PWM, CMV control antigen, CMV, HSV control antigen, or HSV-
1, strain 539, and HSV-2, strain 316-D. The antigen doses used had
been demonstrated previously in preliminary dose-response studies, to
be optimal for the induction of lymphocyte proliferation. The antigen dose
was approximately $7 \times 10^5$ viral plaque-forming units/ml of lymphocyte
culture. Selected cultures were also treated with partially purified IL-2
obtained by venipuncture and defibrinated by swirling with glass beads.

Culture stimulant HSV-1 HSV-2 H-CMV

Table 1 shows the lymphocyte blastogenic responses to the control antigen preparations, the viruses, and the mitogens. The control antigen preparations induced little, if any, blastogenic response. The majority of patients' and control subjects' cells showed some degree of blastogenic response to the virus preparations. The responses of the control subjects' cells to virus were significantly higher than those of the patients only for HSV-1 but not for HSV-2 or for $^3$- CMV. In contrast, the PHA, CON-A, and PWM responses of the control subjects' cells were signifi\cantly higher than those of the patients'. The virus responses were modest, being in the 1000 to 6000 average CPM range, compared to the 30,000 to 60,000 average CPM range for PHA.

Table 2 shows the interferon titers at 24 and 120 hr. Both the patients' and controls' cells showed only a very weak interferon response to CMV. The normal subjects' cells showed a vigorous interferon response to both strains of HSV. It was significantly less by the patients' cells. This was true in both the 24- and 120-hr cultures. Thus, there was a major deficiency in the interferon induction in the patients' cells.

Table 3 shows the relevant NK cell data. Peripheral blood leukocytes were incubated for 4 and 24 hr without any additives and in the presence of viral control antigen or the viral preparations. In general, the patients' unstimulated cells showed signifi

### Table 1

<table>
<thead>
<tr>
<th>Culture stimulant</th>
<th>Patients</th>
<th>Normals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV control antigen</td>
<td>0.32</td>
<td>0.43</td>
<td>NS</td>
</tr>
<tr>
<td>HSV-1</td>
<td>1.75</td>
<td>6.11</td>
<td>0.06</td>
</tr>
<tr>
<td>HSV-2</td>
<td>2.07</td>
<td>6.36</td>
<td>NS</td>
</tr>
<tr>
<td>$^3$-CMV control antigen</td>
<td>0.27</td>
<td>0.48</td>
<td>NS</td>
</tr>
<tr>
<td>$^5$-CMV</td>
<td>3.13</td>
<td>3.38</td>
<td>NS</td>
</tr>
<tr>
<td>Unstimulated control</td>
<td>0.30</td>
<td>0.45</td>
<td>NS</td>
</tr>
<tr>
<td>PHA</td>
<td>38.28</td>
<td>62.05</td>
<td>0.001</td>
</tr>
<tr>
<td>CON-A</td>
<td>13.32</td>
<td>32.35</td>
<td>0.001</td>
</tr>
<tr>
<td>PWM</td>
<td>5.08</td>
<td>22.10</td>
<td>0.002</td>
</tr>
</tbody>
</table>

NS, not significant.
The responses to the virus of the normals' lymphocytes, presum-
and virus-stimulated cultures of the patients. In contrast, it
(Table 4). IL-2 added at the initiation of the cultures markedly
the HSV- and H-CMV-treated cultures, suggesting that NK acti-
cultures treated with IL-2 were only slightly lower than those in
one normal subject was similarly boosted. NK cell activity was
3 patients by at least 2 serial 2-fold dilutions. In contrast, only
not boost the interferon responses of the patients or the normals
ably because these responses were already vigorous. IL-2 did
boost the control antigen-stimulated cultures but did not boost
interferon responses in 4 patients and the HSV-2 responses in
patients, which ranged from 1:64 to 1:4096 with a median of 1:1024, and all but
4 of the patients had an EBV titer which ranged from 1:160 to 1:2560 with a median of 1:640. There was no significant corre-
lion between these titers and the degree of response of the patients to either virus in terms of blastogenesis, NK cell activation, or interferon induction.

**DISCUSSION**

A number of groups have focused studies of AIDS on the
function of various leukocyte subsets. Evidence has already been
developed which suggests that various cytokines, such as IL-2,
may be produced in abnormally low levels by the lymphocytes of
patients with AIDS and that their impaired immunological function in vitro may even be corrected by exogenous administra-
tion of IL-2 (25). While these AIDS patients often manifest an
abnormal acid-labile circulating interferon-α (19), the possibility
exists that they are not capable of producing interferon normally,
since they have a high susceptibility to viral infection. This has
been confirmed in one recent report (14). A generalized failure of
cytokine production might be responsible for the impaired
lymphocyte proliferation, the low levels of NK cells, as well as
the impaired in vivo delayed hypersensitivity.
The data presented in this paper indirectly suggest that exogenous interferon and exogenous IL-2 may be useful in the management of the host-defense failure and opportunistic infections associated with AIDS and ARC disease entities. In order that this objective be achieved, it will be necessary to define carefully the patient subgroups whose cells can or cannot produce these cytokines and to define the interaction between the cytokines simultaneously administered at different in vitro concentrations and their separate and combined in vivo toxicities. The measurement of blastogenic, interferon, and NK responses to these viruses may also be useful in staging and prognosticating for these patients. Finally, observations made in AIDS may have important application to various cancers with an increased susceptibility to viral infection, such as leukemia and lymphoma.

That IL-2 augmented the NK cell response and slightly augmented the interferon response suggests that some interferon-γ production was induced (3). It is known that interferon-γ augments NK cell activity, which can be blocked by the appropriate anti-interferon antibody (24). Another function augmented by interferons, particularly the γ species, is monocyte activation (16) and cytoxicity to virus infection (13) and tumor (15) cells. This poor interferon production itself, or a failure of the cellular defense mechanism directed at virus-infected or -transformed cells, might facilitate the proliferation of a variety of viruses possibly related to AIDS or AIDS-related Kaposi's sarcoma or central nervous system lymphoma (10, 20, 23).

The results reported in this paper indicate that there is indeed impaired, but measurable, lymphocyte proliferation in these patients to the viruses used. Exposure of their lymphocytes to viruses associated with AIDS (such as HCMV and HSV) does not, however, induce normal lymphocyte production (14) and also does not induce normal IL-2 production (25). Failure of production of these 2 cytokines would explain the lack of NK cell boosting in these patients. IL-2 production was not studied concurrently in our patients. Also, since the autologous mixed-lymphocyte reaction generates NK activity (6) and the autologous mixed-lymphocyte reaction is deficient in AIDS (22), it may play a role in the described deficient viral response. Impaired NK cell activation would decrease host control of virus infection and might promote the development of cancer, such as Kaposi's sarcoma and lymphoma. However, their NK cell precursors can clearly still be activated, since addition of exogenous IL-2 induced vigorous NK cell activity in at least some patients.

The data presented in this paper indirectly suggest that exogenous interferon and exogenous IL-2 may be useful in the management of the host-defense failure and opportunistic infections associated with AIDS and ARC disease entities. In order that this objective be achieved, it will be necessary to define carefully the patient subgroups whose cells can or cannot produce these cytokines and to define the interaction between the cytokines simultaneously administered at different in vitro concentrations and their separate and combined in vivo toxicities. The measurement of blastogenic, interferon, and NK responses to these viruses may also be useful in staging and prognosticating for these patients. Finally, observations made in AIDS may have important application to various cancers with an increased susceptibility to viral infection, such as leukemia and lymphoma.


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