IMMUNOLOGICAL STUDIES IN PATIENTS WITH ACQUIRED IMMUNE DEFICIENCY SYNDROME

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INTRODUCTION

Defective cell-mediated immunity in the acquired immune deficiency syndrome (AIDS) is manifested by a reduction of T-helper (T_H) cells and an increase of T-suppressor (T_S) cells in peripheral blood, by loss of normal T-cell responses to mitogens and antigens *in vivo* and *in vitro*, and by increased susceptibility to opportunistic infections and malignancies.[1] Our immunological studies on a group of four patients with AIDS disclosed several preliminary but striking findings that have not previously been reported.

MATERIAL AND METHODS

The patients were homosexual or bisexual males, 30-40 years of age, chosen from a larger group of nine AIDS patients whose blood was submitted to our laboratory for T-cell typing. The patients had histories of Kaposi's sarcoma, opportunistic infections or both, and three of them had neurological complications including cytomegalovirus (CMV) meningitis, cryptococcal meningitis, and cerebral toxoplasmosis. Heparinized blood was collected from patients and controls (laboratory personnel), and mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation. Viability was consistently 90-100 percent. T-cell typing was performed by fluorescence microscopy on aliquots of 10^5 cells using the Leu series of monoclonal antibodies (Becton-Dickinson).

For proliferative studies, cells were incubated for 72 hrs at a density of 10^6/ml in microtiter plates at 37° C in minimal essential medium (MEM) with 20% fetal calf serum (FCS). Proliferation was measured by [^{3}H]thymidine uptake. Cell mixing experiments were carried out by coculturing equal numbers of indicator and modifier cells at a final density of 10^6/ml or 2×10^5 per microtiter well. In some experiments designed to investigate spontaneous suppression, AIDS modifier cells were reduced to three-fifths the usual number to allow for the increased proportion of T_S cells present. Modifier cells were treated with mitomycin C (MC) 25 μg/ml for 30 min at 37° C to inhibit proliferation. Concanavalin A (Con A, Miles-Yeda) was used at 3 μg/ml in all experiments.

Con A-induced suppressor cells were incubated in MEM with 20% FCS and Con A for 96 hrs in 25 cm^2 tissue culture flasks, then treated with MC and washed with

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300 m*M* alpha methyl mannoside before addition to fresh indicator cells. Companion cultures were incubated in MEM and FCS without concanavalin A. After 96 hrs, supernatant fluids were collected, and cells were washed and recultured in fresh medium with addition of Con A to assess changes in proliferative activity. Some of the supernatants were used as conditioned media for fresh indicator cells to assess the antiproliferative effect of a factor released from AIDS cells during incubation. Similarly, fresh sera from one AIDS and two control individuals were added (10% v/v) to cultures of control cells from two donors.

One-way mixed lymphocyte cultures (MLC) were carried out by treating stimulator cells with MC, washing thoroughly, and culturing for 96 hrs with equal numbers (5×10^5) of responder cells, after which proliferation was measured by [^{3}H]thymidine incorporation. Enhancement of AIDS lymphocyte proliferation was also carried out by culturing with equal numbers of MC-treated control cells in the presence of concanavalin A.

Human interleukin-2 (IL-2, Electro-Nucleonics, Inc.) was added (10% v/v) to cultures of control and AIDS lymphocytes without addition of Con A or other mitogens. Responses of IL-2 stimulated and unstimulated cells were measured after 96 hrs *in vitro*.

Results

T-cell subsets in our patients were consistent with the findings of others.[2] By comparison with controls, total T (Leu 4) cells were reduced from 63% to 45%, T_H (Leu 3a+3b) cells were reduced from 44% to 14%, T_S (Leu 2a) cells were increased from 26% to 35%, HLA-DR positive cells were increased from 14% to 25%, and the T_H/T_S ratio was reduced from 1.7 (range 1.0-3.2) in the control group to 0.4 (range 0.1-1.0) in the AIDS group. Mitogen-induced proliferation was also markedly reduced, especially the response to concanavalin A. Proliferation of cells from six controls was 88,580 CPM ± 10,808 (SEM), whereas the mean response of cells from four AIDS patients studied concurrently was 5,671 ± 4,357 counts per minute (CPM).

When MC-treated mononuclear cells from three of the AIDS patients were co-cultured with equal numbers (5×10^5) of cells from normal controls, the responses of the latter to Con A were suppressed to approximately 60% of the normal level. Similar results were obtained when the number of AIDS cells was reduced to compensate for the higher proportion of T_S cells present. Data for three AIDS patients and three different controls are presented in Table 1. Identical experiments carried out using phytohemagglutinin (PHA) instead of Con A revealed only mild (15%) suppression by AIDS cells; hence subsequent experiments were all conducted with concanavalin A.

Because others have shown suppressive factors in sera of AIDS patients,[3,4] an experiment was performed in which 10% control or AIDS sera were added to cultures of control cells in MEM and fetal calf serum. There was only slight (10-23%) suppression of the response compared to the addition of either autologous or homologous normal sera to cultures of the same indicator cells.

Mitomycin C-treated control cells did not suppress proliferation of allogeneic lymphocytes, but rather enhanced their response to concanavalin A. This finding is consistent with the results of others who have shown that normal peripheral blood lymphocytes exert suppressive effects only after preincubation with mitogens or antigens, the most effective inducer of suppression being concanavalin A. Experiments performed to study the susceptibility of AIDS T cells to suppressor induction by Con

TABLE 1

SPONTANEOUS SUPPRESSION OF THE CONCANAVALIN A-INDUCED PROLIFERATIVE RESPONSE BY AIDS LYMPHOCYTES

Cells[a]			
Indicator	Modifier[b]	Δ CPM ± SEM[c]	Percent of Syngeneic Control[d]
Control	Syngeneic Control	65,905 ± 9,878	100
Control	Allogeneic Control	87,940 ± 12,495	117 ± 7
Control	AIDS	44,878 ± 12,208	62 ± 10

[a] 5×10^5 indicator plus 5×10^5 control modifier cells (or 3×10^5 AIDS cells) mixed and incubated in microtiter plates (2×10^5 cell/well) for 72 hrs in MEM plus 20% FCS. Smaller number of AIDS cells used to compensate for higher proportion of T_S cells compared to controls.

[b] Modifier cells preincubated with 25 μg/ml mitomycin C, then washed and adjusted to concentration required for culture.

[c] Δ CPM = CPM of Con A stimulated minus CPM of unstimulated cultures. Values are means ± SEM for three patients in three separate experiments.

[d] Percent change compared to syngeneic controls taken as 100%.

A were inconclusive, resulting in normal (77%) suppression in one patient, moderate (46%) suppression in a second, and no suppression in a third. Normal syngeneic or allogeneic control cells incubated with Con A in the same experiments consistently produced suppression of the Con A response (64-68%).

Although mononuclear cells from AIDS patients responded poorly to Con A, incubation for a period of 96 hrs *in vitro* without mitogenic stimulation restored partial or complete responsiveness. In these experiments the culture media were removed, the cells were washed, then reincubated in fresh medium with FCS and Con A for 72 hours. TABLE 2A shows that the response of control cells was augmented slightly, but the response of cells from three AIDS patients was markedly enhanced (4.9-41.6 times the preincubation values). T helper/T suppressor cell ratios after the preincubation period increased slightly from a mean of 0.3 to a mean of 0.7, with the increase being accounted for mostly by reduction in Leu 2a-bearing cells.

Supernatants from the preincubated cells of two AIDS patients, when transferred as conditioned media to cultures of fresh control cells, suppressed their response to Con A, whereas supernatants from cultures of control cells did not (TABLE 2B), suggesting that suppression was mediated by a soluble factor produced by or bound to the cells and released into the medium. Suppression in this experiment was as effective as that induced by cocultivation of AIDS cells themselves with controls.

Three other methods of augmenting the proliferative response of AIDS lymphocytes were attempted, all resulting in some degree of success. In one-way MLC, cells from this group of patients responded nearly as well as controls to allogeneic stimulation (mean = 3.2× for controls, 3.0× for AIDS). All comparisons were made using the same responder cells. By contrast, AIDS cells were poor stimulators in one-way MLC, with significant stimulation (2.0×) occurring in only one of three experiments. AIDS cells cultured with equal numbers of MC-treated control cells were also shown to respond well to concanavalin A. Using cells from a patient whose proliferative response was 22,364 CPM initially, incubation with two different sets of MC-inactivated control cells resulted in responses of 50,159 and 44,924 CPM respectively, approximately half the normal value for control cells.

Because of recent interest in IL-2 as a possible therapeutic agent for AIDS patients, a single experiment was done using a commercially available product added to cultures

TABLE 2

A. PREINCUBATION OF AIDS LYMPHOCYTES ENHANCES THE PROLIFERATIVE RESPONSE TO CONCANAVALIN A

Subjects	T_H/T_S Ratio	Mean Δ CPM[a]		
		Before	After	Relative Increase
Controls (n = 6)	1.7	88,580	135,225	1.5×
Patient 1	0.1	1,675	21,375	12.8×
Patient 2	0.2	634	26,405	41.6×
Patient 3	0.5	23,029	112,654	4.9×

B. SUPPRESSION OF THE CONCANAVALIN A RESPONSE BY SUPERNATANTS FROM CULTURED AIDS LYMPHOCYTES (TWO PATIENTS)[b]

Cells	Medium	Δ CPM[a]	Percent Suppression
Control	Fresh MEM + FCS	101,491	—
Contol	Control Supernatants	82,891	18%
Control	AIDS Supernatants	45,282	55%

[a] CPM of Con A stimulated minus CPM of unstimulated cultures. Values are means of quadruplicate cultures.

[b] Control or AIDS cells incubated in MEM + FCS without Con A for 72 hrs; supernatants then transferred to cultures of fresh control cells with addition of Con A.

at a concentration of 10% without addition of mitogen. The response of AIDS cells was enhanced 16-fold (631-10,128 CPM) in unstimulated cultures, approximately the same degree to which control responses were enhanced. Studies with the addition of Con A or other mitogens to the IL-2 stimulated cultures remain to be done.

DISCUSSION

T-suppressor cells are characteristically normal or increased in number, whereas T_H cells are drastically reduced in AIDS. The activity of the T_S cells, however, has not been reported except for two studies in which normal[6] or increased[2] suppression of immunoglobulin formation was demonstrated. The present study does not answer this question definitively for the T_S subset, because separated cell populations were not used. The evidence, however, does indicate that a spontaneous cell-mediated suppressive effect on T-cell proliferation occurs in AIDS patients. Not only is there suppression of allogeneic cells *in vitro,* but the same process may be responsible for the failure of AIDS lymphocytes themselves, in particular T_H cells, to proliferate. Incubation of the suppressed cells increases their responsiveness, and probably results in release of a soluble factor into the supernatant fluid, which then has the capacity to suppress proliferation of normal cells. This is the first indication to our knowledge that suppressor cells are abnormally and spontaneously activated in AIDS with respect to their effect on T-cell proliferation. Induction of additional suppression with Con

A in two of three patients suggests that suppressor-activator cells are present, probably in the non-T_S population, despite the marked deficit of T_H cells. These findings suggest the possibility that AIDS may be amenable to treatment by modalities that inhibit, either specifically or nonspecifically, the hyperactive suppressor subset.

Restoration of the proliferative response by preincubation of AIDS cells is also a new finding, though similar results have been reported for patients with CMV mononucleosis,[7] a condition that may be relevant because of the prevalence of CMV infections in AIDS patients. Regeneration of T-cell responsiveness *in vitro* may be due to selective loss of suppressor cells, or possibly to release of a soluble factor that binds to T-cell surface determinants *in vivo*. We favor the latter hypothesis because of previous reports of suppressor factors in AIDS sera[3,4] and the suppressive activity of the supernatants derived from cultures of AIDS cells in our own studies. Our ability to improve proliferative activity of lymphocytes by allogeneic stimulation and by exposure to IL-2 suggests additional methods of enhancing immune responsiveness in patients with AIDS.

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