Decreased suppressor cell activity in disseminated granulomatous infections

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SUMMARY

The effect of granulomatous infections upon the activity of a T lymphocyte subclass in human peripheral blood that can be induced by concanavalin A (Con A) to function in a suppressor mode was studied. Peripheral blood lymphocytes (PBL) from eleven patients with disseminated mycotic or mycobacterial infections or from normal controls were preincubated with and without Con A, washed and cultured with allogeneic PBL freshly drawn from healthy donors sensitive to histoplasmin. DNA synthesis was then measured in co-cultures stimulated by Con A, histoplasmin, or by the mixed lymphocyte culture (MLC) reaction alone. As compared with cells preincubated without Con A, the Con A-pretreated cells from ten of eleven normal donors clearly suppressed the response to Con A by normal allogeneic PBL. Conversely, the response of normal PBL was augmented in six of eleven co-cultures to which Con A-pretreated cells from patients had been added ($P < 0.01$). Likewise, the patients' pretreated cells were significantly less effective in suppressing the responses of normal PBL to histoplasmin ($P < 0.01$), and in a one-way MLC reaction ($P < 0.05$). The Con A-induced suppressor activity of PBL from nine patients with localized granulomatous infections did not differ significantly from that exerted by PBL of normal controls in two of the three co-culture systems employed. These studies suggest that either dysfunction or a reduction of the Con A-inducible T-suppressor cell subpopulation in peripheral blood is frequent among patients with disseminated granulomatous infections.

INTRODUCTION

Exposure of normal human peripheral blood lymphocytes to mitogenic concentrations of concanavalin A (Con A) induces proliferation of a resting T lymphocyte subset that can suppress both antigen-specific and mitogen-stimulated proliferative responses of allogeneic responder cells from healthy donors (Shou, Schwartz & Good, 1976; Hubert, Delespesse & Govaerts, 1976). These findings suggest that the subset of T cells activated to suppressor activity by Con A may play a role in modulating the normal immune response. Support for this concept is provided by recent observations that the Con A-inducible suppressor function of T cells is impaired in patients with active systemic lupus erythematosus (Abdou *et al.*, 1976; Bresnihan & Jasin, 1977). Thus, these patients may be in a state of autoimmune B cell hyperactivity at least in part because of an inability to generate sufficient T-suppressor cell function to modulate B cell activity (Sakane *et al.*, 1979). Conversely, suppressor cell function may be augmented in other diseases. For example, T cells capable of suppressing the pokeweed-induced secretion of immunoglobulin by autologous or

allogeneic B cells are present in the peripheral blood of approximately one-third of patients with common variable acquired hypogammaglobulinaemia (Waldman *et al.*, 1974). Likewise, T lymphocytes have been isolated from the peripheral blood of some patients with disseminated fungal infections that are capable of suppressing proliferative responses to antigens and mitogens by T cells from healthy donors (Stobo *et al.*, 1976).

Persons with disseminated granulomatous infections caused by facultative or obligate intracellular pathogens frequently are anergic when challenged with a battery of recall antigens. Attempts to sensitize them to haptens are unsuccessful in a substantial percentage of cases and the proliferative response of their peripheral blood lymphocytes (PBL) to mitogenic or antigenic stimulation often is impaired (Bullock, 1979). In an attempt to understand better the complex mechanisms by which the cell-mediated immune (CMI) response is depressed, our laboratory is engaged in a series of studies on the immunosuppressor activity of various cell populations in the peripheral blood of patients with disseminated granulomatous infections. In this communication, we report studies designed to test the hypothesis that these infections may effect disturbances in the T lymphocyte subclass that normally can be induced by Con A to manifest suppressor activity *in vitro*. The results demonstrate that as compared with cells from normal donors, there is a significant reduction in the capacity of Con A-pretreated T cells from patients to suppress both the mitogen-stimulated and the antigen-specific proliferative responses of allogeneic responder cells from healthy donors. In contrast, there were no significant differences between the Con A-induced suppressor activity of lymphocytes from patients with *localized* granulomatous infections and normal controls in two of the three test systems employed.

MATERIALS AND METHODS

Patients and controls. Eleven male patients with disseminated infection were studied, ranging in age from 16 to 80 years. As indicated in Table 1, the infecting organisms included *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Mycobacterium tuberculosis* and *Mycobacterium leprae*; patient 4 was doubly infected with *H. capsulatum* and *M. tuberculosis*. One patient infected by *M. leprae* had borderline lepromatous (BL) leprosy as defined by the criteria of Ridley & Jopling (1966) and five had lepromatous (LL) leprosy. In each patient, the pathogen(s) was demonstrated in multiple sites by culture or by histopathological techniques; specimens were obtained from three or more of the following: bronchial secretions, urine, abscess contents, lung, liver, subcutaneous tissue, lymph nodes and bone marrow. No patients had underlying neoplastic or otherwise debilitating diseases and none had received cytotoxic or immunosuppressive therapy prior to study. One patient had a history of alcoholism.

Nine patients were studied who had localized granulomatous infections as determined by extensive laboratory studies and biopsy procedures. Four patients had localized infections caused by *H. capsulatum*, two were infected by *B. dermatitidis*, one by *M. tuberculosis*, one by *Coccidiodes immitis* and one by *M. leprae*.

All except patient 8 were studied prior to or during the first week of antimicrobial therapy.

The control group consisted of healthy subjects (thirteen male and two female) ranging in age from 26–66 years.

Cutaneous delayed-type hypersensitivity (DTH) testing. DTH responses of patients were measured after intradermal (i.d.) challenge with a battery of antigens. Antigens employed included: purified protein derivative (PPD) 5 tuberculin units (Connaught Labs, Ontario, Canada); histoplasmin 1:100 (Parke Davis and Co., Detroit, Michigan); mumps antigen (Eli Lilly and Co., Indianapolis, Indiana); streptokinase–streptodornase (SK/SD), 20 units of SK, 5 units of SD, (Lederle Labs, Pearl River, New York); trichophyton (1:30) and oidomycin (Candida 1:50) (Hollister–Stier Labs, Spokane, Washington). Erythema and induration were measured at 48 hr after injecting 0.1 ml of antigen into the volar aspect of the forearm. Responses were considered positive if induration of at least 5 mm was present (10 mm with SK/SD). In leprosy patients, the skin reaction to integral lepromin (0.1 ml) was measured 3 weeks after injection.

Lymphocyte transformation assay. Peripheral venous blood from patients and controls was

heparinized (20 u/ml, Upjohn Co., Kalamazoo, Michigan), diluted with an equal volume of calcium- and magnesium-free Hanks' balanced salt solution (HBSS), and sedimented by centrifugation at 400 g for 45 min through a lymphocyte separation medium (Litton Bionetics Lab Products, Kensington, Maryland). Cells at the saline-medium interface were removed, washed three times at 270 g for 7 min, and resuspended in RPMI 1640 medium containing 1% penicillin–streptomycin and 1% L-glutamine (GIBCO, Grand Island, New York) and either 15% heat-inactivated pooled human serum (PHS) from healthy donors or 15% heat-inactivated autologous serum. To 2 × 10⁵ cells in 0·1 ml was added 0·1 ml of salt- and carbohydrate-free concanavalin A (Con A; Sigma Chemical Co., St Louis, Missouri), to provide a final concentration of 1·5 μg/well. The concentration of Con A and the duration of culture (5 days) inducing optimal lymphocyte blastogenesis were established previously (see below). Cultures were incubated in Microtest II culture plates (Falcon Plastics, Oxnard, California) for 5 days at 37°C in a humidified atmosphere of 5% CO_2. Sixteen hours prior to cell harvest, 0·5 μCi ³H-thymidine (specific activity 6·7 Ci/mmol, New England Nuclear, Boston, Massachusetts) was added to each culture. Cells were collected on glass-fibre filter pads (Whatman Inc., Clifton, New Jersey) by an automatic sample harvester (Mash II, Microbiological Associates, Bethesda, Maryland) and uptake of radioactivity measured in a liquid scintillation counter. The arithmetic mean of c.p.m. in triplicate samples was determined and the results expressed as the difference between the counts per minute (c.p.m.) of stimulated and unstimulated cultures (Δ c.p.m.).

Con A pretreatment of cells. Suspension cultures of PBL from patients and normal controls were prepared as above, adjusted to a final concentration of 2 × 10⁶/ml and up to 4 ml of suspension placed in 13 × 100 mm round-bottomed glass tubes with loosely fitted screw caps (Bellco Glass Inc., Vineland, New Jersey). One half of the cell suspension prepared from each donor was preincubated with Con A in a final concentration of 60 μg/ml and the other half was preincubated without Con A. All cultures were incubated for 72 hr in a humidified atmosphere containing 5% CO_2. After incubation, the Con A-pretreated and the untreated cells were washed twice in HBSS. The cell suspensions were then irradiated with 3,000 rads using a picker cobalt-60 source and resuspended in RPMI 1640 medium (2 × 10⁶ cells/ml) in preparation for assay of suppressor activity.

Suppressor cell assays. The capacity of cells preincubated with or without Con A from patients and normal controls to suppress mitogen- and antigen-stimulated responses by allogeneic PBL freshly prepared from healthy donors was tested in three separate co-culture systems. In the first system, the fresh responder cells were stimulated by Con A, and in the second, by a recall antigen (histoplasmin). In a third system, the one-way mixed lymphocyte culture (MLC) reaction of the fresh cells to the preincubated cells alone was measured. In all experiments, responder cells were provided by three healthy donors whose PBL consistently displayed vigorous blastogenic transformation when stimulated by Con A or histoplasmin. Each of the co-culture systems was established in triplicate.

To test for induction of suppressor cell activity by Con A, 2 × 10⁵ (0·1 ml) Con A-pretreated cells or cells preincubated without Con A from patients or controls were co-cultured with 2 × 10⁵ responder cells (0·1 ml) in Microtest II culture plates for 5 days at 37°C. In studies of two patients with severe lymphopenia, 1 × 10⁵ preincubated cells were added to an equal number of responder cells. Mitogenic stimulation was provided by adding 0·025 ml of Con A (60 μg/ml) and antigenic stimulation by adding 0·025 ml of a 1 : 10 dilution of histoplasmin HKC-43 (protein content, 585 μg/ml after dialysis to remove preservative). The HKC-43 was provided by Dr Coy Smith, University of Kentucky, Lexington, Kentucky. To the mixed lymphocyte culture system, 0·025 ml of medium alone was added. Sixteen hours prior to harvest, the co-cultures were pulsed with 0·5 μCi ³H-thymidine and the lymphocyte incorporation of isotope-labelled thymidine was measured as described above.

Preliminary studies of suppressor assay methodology. Prior to performing these suppressor assays, studies were performed to determine the necessity of washing Con A-pretreated cells with α-methyl-D-mannoside (αMM) to elute Con A from the cell surfaces before addition to fresh responder cells. In seven separate experiments, Con A-pretreated cells were washed three times with 30 mmol of αMM (Sigma Chemical Co., St Louis, Missouri) in HBSS. Separate aliquots of the pretreated cells were washed three times with HBSS alone. No differences were observed in the

amount of suppression induced by pretreated cells washed with αMM and those washed with HBSS. In three experiments, the optimal concentration of Con A for attempted activation of suppressor activity was determined by dose–response experiments employing concentrations of Con A ranging from 10 to 80 μg/ml. In each experiment, the optimal concentration of Con A for induction of suppressor activity was 60 μg/ml. Additional experiments were performed to study the effect of preincubating PBL with Con A for 24, 48, 72 and 96 hr. Maximum suppressor activity was observed at either 48 or 72 hr of Con A preincubation; a 72-hr preincubation period was employed for all studies. Finally, the optimum concentration of Con A (1·5 μg/well) to be employed for stimulation of fresh responder cells in the suppressor assay was determined by constructing dose–response curves subsequent to experiments with concentrations of Con A ranging from 0·5 to 6·0 μg/well. The optimal concentration of histoplasmin (1 : 10) was established by similar dose–response experiments.

Evaluation of per cent suppression. The per cent suppression induced by Con A-pretreated cells or cells preincubated without Con A on the response of fresh allogeneic cells to mitogenic or antigenic stimulation was calculated according to the following formula:

$$1 - \frac{\substack{(\text{mean c.p.m. of stimulated co-cultures containing Con A-pretreated cells}) - \\ (\text{mean c.p.m. of unstimulated co-cultures containing Con A-pretreated cells})}}{\substack{(\text{mean c.p.m. of stimulated co-cultures containing control cells}) - (\text{mean c.p.m. of} \\ \text{unstimulated co-cultures containing control cells})}} \times 100.$$

The effect of Con A pretreatment on the MLC response was calculated according to the following formula:

$$1 - \frac{(\text{mean c.p.m. of co-cultures containing Con A-pretreated cells})}{(\text{mean c.p.m. of co-cultures containing control cells})} \times 100.$$

In each formula, the term 'control cells' refers to cells preincubated without Con A.

T and B lymphocyte quantitation. The percentage of E rosette-forming cells (T cells) in the peripheral blood of controls and patients was estimated by the method of Jondal, Holm & Wigzell (1972). Lymphocytes binding more than three sheep red blood cells (SRBC) were considered to be rosette-forming cells. The percentage of B cells was determined by direct immunofluorescence microscopy using the F(ab')$_2$ fragment of goat antibody to human serum conjugated with fluoroscein–isothiocyanate (Cappel Labs Inc., Cochranville, Pennsylvania) according to the method of Pernis, Forni & Amante (1970). Cell preparations were examined with a fluorescence microscope equipped with an Osram HBO-200 mercury vapour lamp and a vertical epi-illuminator. In each experiment, 500 lymphocytes were counted.

Statistical methods. Differences between groups were analysed by the Wilcoxon sum of the rank test for paired samples or the Mann–Whitney test using a two-sided alternative to determine significant differences between groups (Snedecor & Cochran, 1967).

RESULTS

Clinical profile of patients

The clinical profiles of eleven patients with disseminated granulomatous infections are summarized in Table 1 (see Materials and Methods section). All five patients with disseminated fungal infections or tuberculosis were anergic to cutaneous challenge with multiple recall antigens. Each of the six leprosy patients failed to respond to skin testing with lepromin and three were anergic to an entire antigen test battery. Patient 8 had received dapsone therapy for 2 years and the status of his reactivity prior to therapy was unknown. Patients with fungal infection were treated with from 1·5 to 2·5 g of amphotericin B and those with mycobacterial infections were treated with various drug combinations including isoniazid, ethambutol, streptomycin, rifampicin, dapsone and thalidomide. In ten of eleven patients, no relapses have been observed during follow-up periods of from 1 to 2 years. Necropsy of patient 2 demonstrated disseminated histoplasmosis after therapy with ampho-

Table 1. Clinical profile of eleven patients with disseminated granulomatous infections

Patient	Age (years)	Infecting organism	Skin responses*	Therapy†	Outcome
1	51	*B. dermatitidis*	0/5	A	Arrested
2	56	*H. capsulatum*	0/5	A	Expired
3	48	*H. capsulatum*	0/5	A	Arrested
4	57	*H. capsulatum*			
		M. tuberculosis	0/5	A, E, I, S	Arrested
5	53	*M. tuberculosis*	0/5	E, I, R	Arrested
6	80	*M. leprae*	0/5	D	Improved
7	16	*M. leprae*	0/5	D, T	Improved
8	63	*M. leprae*	1/5	D, T	Improved
9	23	*M. leprae*	0/5	D	Improved
10	58	*M. leprae*	2/5	D, T	Unchanged
11	51	*M. leprae*	1/5	D	Improved

* Number of positive skin tests over number of skin tests applied.

† A = amphotericin B; D = dapsone; E = ethambutol; I = isoniazid; R = rifampicin; S = streptomycin; T = thalidomide.

tericin B (to a total dosage of 2·5 g). It was possible to reapply a skin test battery to patients 1, 4 and 5 approximately 6 months after completion of therapy. In each case, there was recovery of skin test reactivity to antigens of the infecting agent as well as to other recall antigens.

Quantitation of PBL

Absolute lymphocyte counts in the blood of patients with disseminated infection ranged from 664 to 2,130/mm^3 with a median of 1,443/mm^3. These values differed significantly ($P < 0.05$) from the absolute lymphocyte counts of normal controls established in our laboratory (median 2,128/mm^3; range, 1,056 to 3,264/mm^3). The median percentage of circulating T cells in the patient group was 44·5% (range, 27·7 to 66·7%) and 59·0% (range, 50 to 67%) in the normal controls ($P < 0.02$). The median percentage of B lymphocytes in the blood of patients was 21·7% (range, 12·3 to 38·0%) and in the blood of healthy controls the percentage of B cells was 15·7% (range, 10 to 21%). The difference was not significant ($P < 0.10$).

Lymphocyte blastogenic response to Con A

In paired experiments, the PBL from nine patients and normal control donors were washed and cultured in medium containing either a 15% concentration of pooled serum (heat-inactivated) from normal donors or 15% serum from the infected patient. As shown in Table 2, the response to Con A by PBL from patients was significantly lower than that by PBL from normal donors when these cells were cultured in medium containing normal serum ($P < 0.01$). Likewise, the difference between background counts and counts generated in response to Con A (Δ c.p.m.) was significantly lower in cultures of PBL from patients as compared with controls ($P < 0.01$). In these experiments, the differences between groups are not reflected in the median values but are readily seen in the ranges of values. When PBL were cultured in medium containing patient serum, the responses to Con A by cells from patients were suppressed severely as compared with the response of cells from normal donors ($P < 0.01$). This was true whether the results were expressed as total c.p.m. or Δ c.p.m. Responses to Con A by normal PBL were not significantly lower in patient serum medium than in normal serum medium. Conversely, the PBL of patients responded very poorly in medium containing autologous serum as compared with medium containing normal serum.

Effect of Con A-pretreated PBL upon the response to Con A by normal allogeneic cells

As compared with aliquots of PBL preincubated as controls for 72 hr without Con A, the Con A-pretreated PBL from normal donors clearly suppressed blastogenic responses to Con A by

Table 2. Proliferative responses to Con A by peripheral blood lymphocytes (PBL) from patients with disseminated infection and normal controls

| Response measured	Normal serum		Patient serum	
	Normal PBL (c.p.m.)	Patient PBL (c.p.m.)	Normal PBL (c.p.m.)	Patient PBL (c.p.m.)
Unstimulated cultures	378*	1,036	212	381
	(159–1,100)†	(169–2,923)‡	(83–637)	(127–788)
Con A-stimulated cultures	31,139	30,148	29,338	7,000
	(22,399–96,660)	(5,750–52.854)‡	(6,700–67,083)	(749–9,964)‡
Δ c.p.m.	30,761	28,651	47,764	6,315
	(22,120–96,398)	(4,290–52,513)‡	(22,971–70,678)	(19–9,485)‡

* Median value.
† Figures in parentheses indicate range of values.
‡ Significant difference between patient and normal control group ($P < 0.01$).

allogeneic PBL from healthy individuals in ten of eleven experiments. In Fig. 1 are shown the results of individual co-culture experiments that range from 35% augmentation of the response to Con A to 73% suppression with a median value of 31% depression. By contrast, the suppressive effect of Con A-pretreated PBL from patients with disseminated infection was significantly less than that of pretreated cells from normal donors ($P < 0.01$). The maximum suppression induced by Con A-pretreated cells from any patient was 21%. In fact, the cells from six of eleven patients actually augmented the response to Con A, in one case by 655%. Although Con A-pretreated lymphocytes from five patients did weakly suppress responses to Con A by normal cells, the median value for all cultures was 5% augmentation of the response to Con A. (Fig. 1).

Effect of Con A-pretreated PBL upon the response to histoplasmin by normal allogeneic cells
A second set of experiments was performed to determine the effect of Con A-pretreated cells from patients and normal controls upon the responses to antigenic stimulation by allogeneic cells from
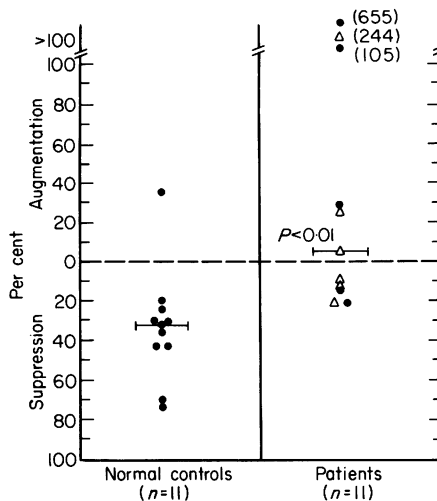
Fig. 1. Effect of Con A-pretreated cells from patients or normal controls on responses to Con A by allogeneic cells from normal donors. Median values are indicated by horizontal bars; (△) leprosy patients.

healthy donors. Histoplasmin was employed as the stimulus for blastogenic transformation of cells obtained from donors known to express DTH to this antigen preparation. As shown in Fig. 2, Con A-pretreated cells from normal donors suppressed the responses to histoplasmin in all experiments. The median value of suppression was 33% with a range of 2 to 96%. Conversely, the suppression of responses to histoplasmin by Con A-pretreated cells from patients was significantly less ($P < 0.01$). The median value of suppression was 10% whereas the range of values extended from augmentation of 148% to a maximum suppression of 47%.

Effect of Con A-pretreated PBL on the MLC reaction

Con A-pretreated cells for normal donors severely suppressed the one-way MLC reaction of the normal allogeneic responder cells (median 42%; range 9 to 95%). On the other hand, significantly less suppression of the MLC was induced by Con A-pretreated cells from the patient group ($P < 0.05$) as seen in Fig. 3. The results of the experiments with patients' cells appeared to be arrayed in a bimodal pattern with a subset of five patients whose Con A-preincubated cells greatly enhanced the one-way MLC response whereas pretreated cells from the other six patients effectively suppressed the MLC reaction by normal cells. The median suppression for all eleven experiments was 10% with the range extending from 300% augmentation to 77% suppression.

Suppressor effects of Con A-pretreated cells from patients with localized infection

Additional experiments were performed with PBL from patients with localized granulomatous infections caused by pathogenic fungi or Mycobacteria. The results of these studies are summarized in Fig. 4. The Con A-pretreated cells from these patients did not differ significantly from the pretreated cells from healthy donors in their ability to suppress the response of normal allogeneic cells to Con A ($P > 0.10$). The median value of suppression was 18% with a range of values extending from 14% augmentation to 90% suppression. Likewise, the magnitude of suppression of responses to histoplasmin induced by cells from patients with localized infections did not differ significantly from that induced by pretreated cells from normals (median suppression 22%; range 1 to 53%). In studies of the MLC reaction, however, pretreated PBL from patients clearly were less suppressive ($P < 0.01$) than pretreated cells from healthy donors. The median response in these experiments was 1% augmentation with a range from 80% augmentation to 43% suppression.
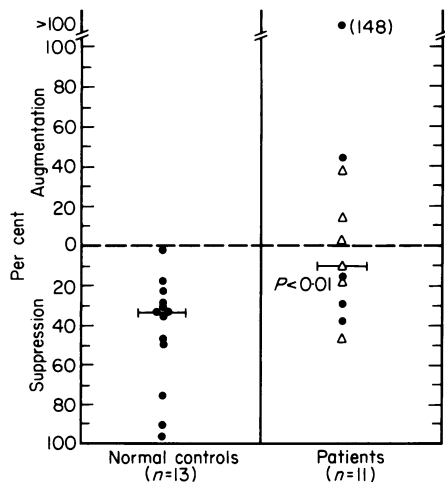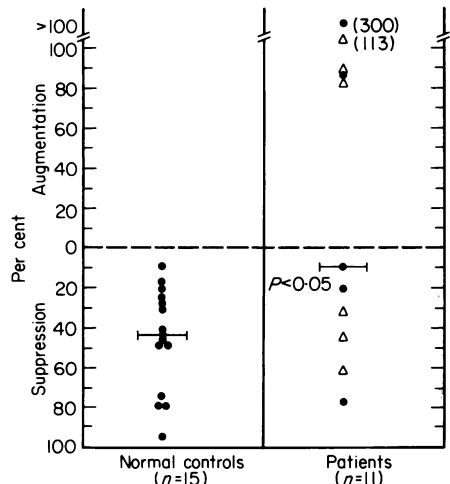

Fig. 2. Effect of Con A-pretreated cells from patients or normal controls upon responses to histoplasmin by allogeneic cells from normal donors. Symbols as in Fig. 1.

Fig. 3. Effect of Con A-pretreated cells from patients or normal controls on one-way mixed lymphocyte culture (MLC) reactions of allogeneic cells from normal donors. Symbols as in Fig. 2.
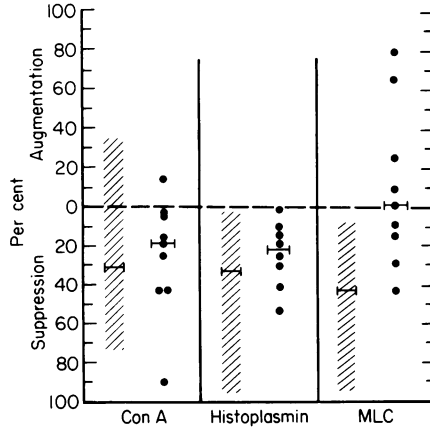
Fig. 4. Effect of Con A-pretreated cells from patients with localized disease on response by cells from normal donors to Con A, histoplasmin or mixed lymphocyte stimulation. Hatched areas on the left of each sector indicate the range of suppression induced in previous experiments by normal cells pretreated with Con A; (●) results of individual experiments with cells from patients. Horizontal bars denote median values.

DISCUSSION

These studies have demonstrated that there is a reduction in the Con A-inducible suppressor activity of the PBL from patients with disseminated granulomatous infections. Although Con A-pretreated PBL from some patients could be induced to function in a suppressor mode, the PBL from others failed completely to suppress the responses to allogeneic PBL from healthy donors to either mitogenic or antigenic stimulation. In fact, addition of Con A-pretreated PBL from patients to normal lymphocytes actually augmented blastogenic responses by the latter cells in six of eleven experiments. Similar augmentation was observed in two additional sets of experiments wherein normal allogeneic PBL were stimulated by histoplasmin antigen or alloantigens respectively. In each set, the Con A-pretreated cells from five of eleven patients augmented the response of normal lymphocytes to these antigens (Figs 2 and 3). On the other hand, Con A-pretreated cells from patients with localized granulomatous infections were able to suppress the responses of normal PBL to stimulation by both Con A and histoplasmin although suppression of the responses to alloantigens was significantly less than normal. Thus, the function of the Con A-inducible suppressor T cell subset clearly was less disturbed in patients with localized granulomatous infections.

The mechanism(s) by which Con A-induced suppressor cell activity is decreased in patients with disseminated granulomatous infection is speculative. Since the absolute numbers of lymphocytes and the percentages of T cells were significantly below normal in the peripheral blood of these patients, the Con A-inducible suppressor T cell subset may have been reduced in proportion to all other T cells. Conceivably, a selective loss of Con A-inducible suppressor cells could have accounted for the net reduction in total T cells. It should be noted, however, that the blastogenic responses to Con A by PBL from these patients were reduced significantly as compared with normal controls. Given this finding, plus recent evidence that the Con A-inducible suppressor subpopulation is composed of high-density T lymphocytes exhibiting a *poor* blastogenic response to Con A (Sakane & Green, 1977), it is very unlikely that selective loss of these cells accounted for the reduction in responses to Con A. The present studies do not exclude the possibility that in patients, the subset of cells normally triggered to suppressor activity by Con A were hyporesponsive to suppressor induction.

One possible mechanism by which T lymphocytes and subpopulations thereof may be reduced in the blood of persons with disseminated granulomatous infections is entrapment of recirculating T cells within the spleen and lymph nodes as has been demonstrated to occur in mice with experimental mycobacterial infections (Bullock, 1976a, b). Alternatively, cytotoxic autoantibodies to T

cell subsets may be present during active disease as is the case in certain connective tissue diseases (Strelkauskas *et al.*, 1978; Sakane *et al.*, 1979). Antibodies cytotoxic to lymphocytes were not detected in the subjects of this study (Bullock, unpublished observations). Nevertheless, the finding of cold lymphocytotoxins in the serum of some leprosy patients by Kreisler *et al.* (1975) suggests that longitudinal studies are needed to monitor for the presence of antibodies to lymphocytes in the serum of persons with granulomatous infections.

A reduction in activity of the Con A-inducible suppressor T cell subset may have permitted greater expression of helper cell function thus augmenting many of the immune responses in the assays employed. Conversely, the disseminated infections could have stimulated helper cell sub-populations to unusually high levels of activity thereby masking suppressor cell activity. In preliminary studies, we have attempted to detect extraordinary helper cell activity by measuring the effect of adding freshly drawn PBL from patients to normal PBL that are stimulated with Con A. The responses of the normal cells in these co-cultures tended to be somewhat higher than in control cultures to which fresh cells from healthy donors had been added but the results did not differ significantly (Artz & Bullock, unpublished observations). Thus, putative hyperactivity of helper cells as a consequence of chronic stimulation by microbial antigens *in vivo* does not appear to provide sufficient explanation for the findings of this study.

A final postulate as to the cause of the diminished suppressor activity observed is suggested by recent work of Raff, Cochrum & Stobo (1978) who demonstrated that low-density cells of the monocyte–macrophage series bearing the human equivalent of murine Ia antigen are required accessory cells for generation of Con A-inducible suppressor T cells. Since the inflammatory response to many obligate or facultative intracellular pathogens is comprised largely of macro-phages, it is worth exploring the thesis that subsets of monocytes capable of accessory function in Con A induction of suppressor T cells may be depleted from the peripheral blood of these patients or may exhibit intrinsic functional impairment.

Functional impairment of one suppressor T cell subpopulation by no means precludes the presence of other T cell subsets in peripheral blood that do function actively in the suppressor mode. Indeed, a short-lived subpopulation of suppressor T cells has been detected in the peripheral blood of some patients with disseminated fungal infections by Stobo *et al.* (1976). These cells act non-specifically to suppress T-proliferative responses to both antigens and mitogens. Furthermore, Bjune (1979) and Mehra *et al.* (1979) reported that the proliferative response to phytohaemagglu-tinin or Con A by PBL from patients with lepromatous leprosy is reduced significantly in the presence of *M. leprae* antigens as compared with lymphocyte cultures to which these antigens have not been added. Thus, *M. leprae* antigens may induce a T cell subpopulation to suppress non-speci-fically the response to mitogens by other T cell subsets. It is unknown what significance, if any, these respective suppressor cell subsets may have with respect to the anergy and poor clinical responses frequently observed among patients with disseminated granulomatous infections.

Decreased Con A-inducible suppressor cell activity very similar to that seen in our patients has been described in persons with chronic active hepatitis (Hodgson, Wands & Isselbacher, 1978a) and inflammatory bowel disease, i.e. ulcerative colitis or Crohn's disease (Hodgson, Wands & Issel-bacher, 1978b). Common to these conditions is a state of persistent inflammation that not infrequently is associated with polyclonal hypergammaglobulinaemia and autoantibody forma-tion. These respective abnormalities may be secondary to non-specific stimulation of the lymphoid system by chronic infection and autoantibody formation in response to cellular antigens released by a persistent inflammation. On the other hand, our study and others cited above suggest that the feedback control mechanisms in some patients with these diseases in fact may be disturbed to the point that they fail to modulate T and B cell interactions adequately.

It is important therefore to determine if the altered Con A-induced suppressor activity asso-ciated with systemic granulomatous pathology is merely an epiphenomenon or is of biological importance. This can be achieved only by prospective studies using techniques that permit func-tional and numerical evaluation of T cell subsets with specific cell markers, and by elucidation of subset dynamics throughout the clinical course of disease.

REFERENCES

ABDOU, N., SAGAWA, A., PASCUAL, E., HEBERT, J. & SADEGHEE, S. (1976) Suppressor T-cell abnormality in idiopathic systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **6,** 192.

BJUNE, G. (1979) *In vitro* lymphocyte stimulation in leprosy: simultaneous stimulation with *Mycobacterium leprae* antigens and phytohaemagglutinin. *Clin. exp. Immunol.* **36,** 479.

BRESNIHAN, B. & JASIN, H.E. (1977) Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. clin. Invest.* **59,** 106.

BULLOCK, W.E. (1979) Mechanisms of anergy in infectious diseases. In *Immunological Aspects of Infectious Diseases* (ed. by G. Dick). University Park Press, Baltimore.

BULLOCK, W.E. (1976a) Perturbation of lymphocyte circulation in experimental murine leprosy. I. Description of the defect. *J. Immunol.* **117,** 1164.

BULLOCK, W.E. (1976b) Perturbation of lymphocyte circulation in experimental murine leprosy. II. Nature of the defect. *J. Immunol.* **117,** 1171.

HODGSON, H.J.F., WANDS, J.R. & ISSELBACHER, K.J. (1978a) Decreased suppressor cell activity in inflammatory bowel disease. *Clin. exp. Immunol.* **32,** 451.

HODGSON, H.J.F., WANDS, J.R. & ISSELBACHER, K.J. (1978b) Alteration in suppressor cell activity in chronic active hepatitis. *Proc. Natl. Acad. Sci. USA,* **75,** 1549.

HUBERT, C., DELESPESSE, G. & GOVAERTS, A. (1976) Concanavalin A-activated suppressor cells in normal human peripheral blood lymphocytes. *Clin. exp. Immunol.* **26,** 95.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. exp. Med.* **136,** 207.

KREISLER, J.M., ARNAIZ, A., PEREZ, B. & BOOTELLO, A. (1975) Lymphocytotoxins in leprosy. *Int. J. Lepr.* **43,** 91.

MEHRA, V., MASON, L.H., FIELDS, J.P. & BLOOM, B.R. (1979) Lepromin-induced suppressor cells in patients with leprosy. *J. Immunol.* **123,** 1813.

PERNIS, B., FORNI, L. & AMANTE, L. (1970) Immunoglobulin spots on the surface of rabbit lymphocytes. *J. exp. Med.* **32,** 1001.

RAFF, H.V., COCHRUM, K.C. & STOBO, J.D. (1978) Macrophage–T cell interactions in the Con A induction of human suppressive T cells. *J. Immunol.* **121,** 2311.

RIDLEY, D.S. & JOPLING, W.H. (1966) Classification of leprosy according to immunity. *Int. J. Lepr.* **34,** 255.

SAKANE, T. & GREEN, I. (1977) Human suppressor T cells induced by concanavalin A: suppressor T cells belong to distinctive T cell subclasses. *J. Immunol.* **119,** 1169.

SAKANE, T., STEINBERG, A.D., REEVES, J.P. & GREEN, I. (1979) Studies of immune functions of patients with systemic lupus erythematosus. *J. clin. Invest.* **63,** 954.

SHOU, L., SCHWARTZ, S.A. & GOOD, R.A. (1976) Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. *J. exp. Med.* **143,** 1100.

SNEDECOR, G.W. & COCHRAN, W.G. (1967) *Statistical Methods* 6th edn. The Iowa State University Press, Ames.

STOBO, J.D., PAUL, S., VAN SCOY, R.E. & HERMANS, P.E. (1976) Suppressor thymus-derived lymphocytes in fungal infection. *J. clin. Invest.* **57,** 319.

STRELKAUSKAS, A.J., CALLERY, R.T., MCDOWELL, J., BOREL, Y. & SCHLOSSMAN, S.F. (1978) Direct evidence for loss of human suppressor cells during active autoimmune disease. *Proc. Natl. Acad. Sci. USA,* **75,** 5150.

WALDMAN, T.A., BRODER, S., BLAESE, R.M., DURM, M., BLACKMAN, M. & STROBER, W. (1974) Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinemia. *Lancet,* **ii,** 609.