Immunomodulatory Effect of Procainamide in Man

INHIBITION OF HUMAN SUPPRESSOR T-CELL ACTIVITY IN VITRO

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ABSTRACT Procainamide (PA) induces the production of a number of autoantibodies in a high proportion of treated individuals and in some a syndrome closely resembling systemic lupus erythematosus. The mechanism underlying this action of PA is unclear. To examine the possibility that PA might induce autoantibody formation by altering normal immunoregulatory mechanisms, the action of this drug on an in vitro model of antibody formation in man was examined. PA was found to augment the generation of immunoglobulin-secreting cells (ISC) from human peripheral blood mononuclear cells (PBM) in response to pokeweed mitogen but had no effect on pokeweed mitogen-induced tritiated thymidine incorporation. When purified populations of B and T cells were used, PA enhanced the generation of ISC in B-cell cultures supported by untreated T cells but not by T cells treated with mitomycin C. These results indicate that PA augmented B-cell responses by inhibiting suppressor T-cell activity and not by augmenting helper T-cell or B-cell function. *N*-Acetyl-procainamide had no effect on the generation of ISC in this system.

The effect of PA on concanavalin A (Con A)-induced suppressor cell activity was also examined to determine whether PA altered the generation or expression of suppressor T-cell function. PBM were cultured with 30 μg/ml of Con A for 48 h to generate suppressor cells. When these were co-cultured with fresh PBM, the number of ISC generated was decreased by 58.1±3.4% (mean ±SEM, $n = 6$). Cells that had been similarly incubated without Con A were not inhibitory. The addition of PA to the Con A-stimulated cultures inhibited the generation of suppressor cells as indicated by the fact that the response of fresh cells co-cultured with the Con A-stimulated cells was diminished by only 27.2±4.3%. In this system too, *N*-acetyl-procaimamide had no effect. By contrast, adding PA only to the co-culture of Con A-stimulated cells with fresh PBM had a less marked effect on suppressor cell function. These results indicate that the major action of PA is to inhibit the generation of suppressor T-cell activity. Such an effect may explain the capacity of this agent to induce autoantibody formation in treated individuals.

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INTRODUCTION

The production of various autoantibodies indicative of a state of autoimmunity has been observed commonly in patients treated with procainamide (PA)[1] for cardiac arrhythmia (1–6). Prospective studies have revealed that PA may induce antinuclear antibodies in 50–83% of individuals, and that 12–29% of PA-treated patients develop a syndrome resembling systemic lupus erythematosus (SLE) (7–10).

The development of autoimmunity induced by PA is influenced by factors that affect the serum level of this agent. These include dosage (2), duration of administration (8), and rate of acetylation of the parent drug (11). Thus, individuals who are slow acetylators maintain higher serum levels of PA and develop antinuclear antibodies sooner than those who are fast acetylators (11). Consistent with these findings is the recent observation of Kluger et al. (12) that *N*-acetyl-procainamide (NAPA), which is deacetylated in vivo only to a very limited degree, does not cause a recurrence of symptoms in most patients who previously had developed PA-induced SLE.

The mechanism underlying the development of autoimmunity in PA-treated patients remains controversial. In sera from patients with PA-induced SLE, an-

[1] Abbreviations used in this paper: Con A, concanavalin A; IgG SC, IgG-secreting cells; IgM SC, IgM-secreting cells; ISC, immunoglobulin-secreting cells; NAPA, *N*-acetyl-procainamide; PA, procainamide; PWM, pokeweed mitogen; SLE, systemic lupus erythematosus; SPA, *Staphylococcus aureus* protein A; [^{3}H]Tdr, tritiated thymidine.

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tibodies to histone (5) and denatured DNA (3) have been detected. It has been proposed that chemical interaction between PA and nucleoproteins enhances the immunogenicity of the nucleoproteins (4, 13) and that interactions between drug and DNA produce drug-DNA complexes that predispose to autoantibody formation and in this way play a pathogenetic role in PA-induced SLE. On the other hand, Bluestein et al. (6) reported that the spectrum of autoantibodies found in PA-induced SLE was not limited to antibodies directed at nuclear antigens but that high titers of cold-reactive lymphocytotoxic antibodies to normal human lymphocytes could also be found. Moreover, these authors found that pharmacologically attainable concentrations of PA enhanced the mitogenic response of normal peripheral blood mononuclear cells (PBM) to phytohemagglutinin in vitro. On this basis, an alternative hypothesis was suggested (6, 14), i.e., that PA might interact directly with the lymphocyte membrane leading to a defect in immunoregulation and thus predispose to the development of autoimmunity.

The studies presented in this paper examine the effect of PA on an in vitro model of human antibody formation. The results indicate that PA, but not NAPA, exerts an inhibitory effect on the generation of suppressor T cells. These results provide evidence that PA may stimulate the development of autoimmunity by interfering with the normal immunoregulatory role of suppressor T cells.

METHODS

Reagents. Crystallized PA and NAPA were kindly provided by Dr. Robert Lahita of the Rockefeller University, New York.

Culture medium. All cultures were carried out in RPMI 1640 medium (Microbiological Associates, Walkersville, MD), supplemented with penicillin G (200 U/ml), gentamicin (10 μg/ml), L-glutamine (0.3 mg/ml), and 10% fetal bovine serum (Microbiological Associates).

Cell preparation. PBM were obtained from normal adult volunteers by centrifugation of heparinized venous blood on sodium diatrizoate/Ficoll cushions (15).

Cell separation. PBM were depleted of adherent cells by two successive incubations on glass petri dishes (15).

To obtain T- and B-cell-enriched populations, glass nonadherent cells (NAC) were rosetted with neuraminidase-treated sheep erythrocytes (N-SRBC) (16), followed by centrifugation on diatrizoate/Ficoll cushions. The interface cells were harvested, again rosetted with N-SRBC to remove residual T cells and used as a B-cell-enriched population. The pelleted cells were treated with isotonic NH_4Cl, passed over a nylon wool column (17), washed three times with Hanks' balanced salt solution (HBSS) and used as the T-cell-enriched population.

In some experiments, T cells were treated with mitomycin C (Sigma Chemical Co., St. Louis, MO) before culture (17). This was accomplished by suspending the T cells in HBSS at a concentration of $\sim 5 \times 10^6$/ml and incubating them on a rotator for 45 min at 37°C with mitomycin C at a concentration of 40 μg/ml. Afterward, the cells were washed four times and suspended in culture medium.

Culture conditions for generation of immunoglobulin-secreting cells (ISC). Cultures were carried out in triplicate in round-bottom microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) with each microwell containing 1×10^5 PBM in 0.2 ml of culture medium. As stimulus, pokeweed mitogen (PWM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) at a previously determined optimal concentration of 10 μg/ml, *Staphylococcus aureus* protein A (SPA, Pharmacia Fine Chemicals, Uppsala, Sweden) at a concentration of 1 μg/ml, or the same volume of HBSS as control was added to each well. When purified B and T cells were cultured, each well contained 2.5×10^4 B cells and various numbers of control or mitomycin C-treated T cells. After incubation for 6 d at 37°C in a humidified atmosphere of 5% CO_2 and 95% air, cells from triplicate wells were pooled, washed, and resuspended in HBSS for enumeration of ISC.

Enumeration of ISC. ISC were enumerated by a reverse hemolytic plaque assay (18, 19) that used SPA-coated sheep erythrocytes, polyvalent rabbit antiimmunoglobulin developing antiserum (anti-IgM + anti-IgG + anti-IgA, N. L. Cappel Laboratories, Inc., Cochranville, PA) and a 1:20 dilution of guinea pig serum (Pel-Freez Biologicals, Inc., Rogers, AR) that had been absorbed previously with SRBC as a source of complement. To detect IgM-secreting cells (IgM SC) and IgG-secreting cells (IgG SC) individually, monospecific rabbit anti-IgM and anti-IgG antisera (Miles Laboratories, Inc., Elkhart, IN) were used. Data were normalized by computing the number of ISC per 10^6 responding cells initially cultured. For each experimental point (consisting of cells pooled from triplicate culture wells), replicate ISC determinations were carried out. Data presented are the mean number ISC and variability about the mean was always <10%.

Generation of suppressor cells by concanavalin A (Con A). PBM (5×10^6 cells/ml) were cultured with or without Con A (Pharmacia Fine Chemicals) at a concentration of 30 μg/ml for 48 h at 37°C in petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) on a rocker platform with shaking. To examine the effect of the drugs under study, PA, NAPA, or HBSS as a control was added to both Con A-activated and control cells at the start of the 48-h culture. As control, Con A was added to the nonactivated control cells just before harvest. Both Con A-activated cells and nonactivated control cells were then washed three times with HBSS supplemented with 0.06 M α-methyl-D-mannoside (20) (Sigma Chemical Co.) and two times in HBSS and then resuspended in culture medium to examine their effect on the generation of ISC.

Preparations of Con A-activated cells or nonactivated control cells were co-cultured with fresh PBM (responder cells) in microtiter wells to assay for suppressor cell activity. In this assay culture, the total number of cells cultured per microwell was kept constant (1×10^5/well), while the proportion of the individual cell populations was varied. PWM (10 μg/ml) or an equivalent volume of HBSS as control was added to the wells and they were incubated for 6 d in standard fashion before enumeration of ISC.

Percent suppression of the generation of ISC was calculated from the formula:

$$\% \text{ suppression} = \left[1 - \frac{\text{ISC observed}}{\text{ISC predicted}}\right] \times 100.$$

The predicted number of ISC is derived by calculating the sum of the products obtained by multiplying the number of

ISC generated when each population of cells is cultured alone by the relative proportion of the co-culture made up by that population.

Assay of DNA synthesis. PBM or NAC were cultured in triplicate in microtiter plates with U-bottomed wells in identical fashion to that used for the generation of ISC. 4–6 h before harvesting, 1 μCi of tritiated thymidine ([^{3}H]Tdr, 6.7 Ci/mM, New England, Boston, MA) was added to each well. After a total incubation of 72 h, the cells were harvested onto glass fiber filter paper using a multiple sample automated harvester (Mash II, Microbiological Associates). [^{3}H]Tdr incorporation was measured by liquid scintillation spectroscopy. All data are expressed as the difference in counts per minute between the means of triplicate mitogen stimulated and control cultures (Δcpm).

RESULTS

Effect of PA on mitogen responsiveness of human PBM. To determine whether PA altered human lymphocyte responsiveness, PBM were cultured with various concentrations of PA or NAPA in the presence of PWM and assayed for [^{3}H]TdR incorporation and the generation of ISC. Since the plasma concentration of PA in treated patients may be as much as 30 μg/ml (21, 22), concentrations of PA and NAPA between 10 and 40 μg/ml were examined. Typical results are shown in Fig. 1. Neither PA nor NAPA had any significant effect on PWM-induced [^{3}H]TdR incorporation at concentrations up to 40 μg/ml. However, there was marked augmentation of ISC generation at concentrations ≥ 20 μg/ml of PA. NAPA caused no similar increase in responsiveness. In most experiments, 30 μg/ml PA produced maximal augmentation of the generation of ISC and this concentration was therefore used in subsequent experiments. In none of the experiments did PA have an effect on the generation of ISC in cultures not stimulated by mitogen. Table I shows the mean results obtained when PBM from 10 different healthy donors were examined. PA had a significant enhancing effect on the ISC response ($P < 0.02$, determined by Student's t test) but no significant effect on [^{3}H]Tdr incorporation stimulated by PWM. On the other hand, PA had no significant enhancing effect on ISC or [^{3}H]Tdr incorporation stimulated by SPA. In additional experiments not shown, PA at concentrations as high as 30 μg/ml was also found to have no significant effect on [^{3}H]Tdr incorporation induced by Con A after 72 h of incubation.

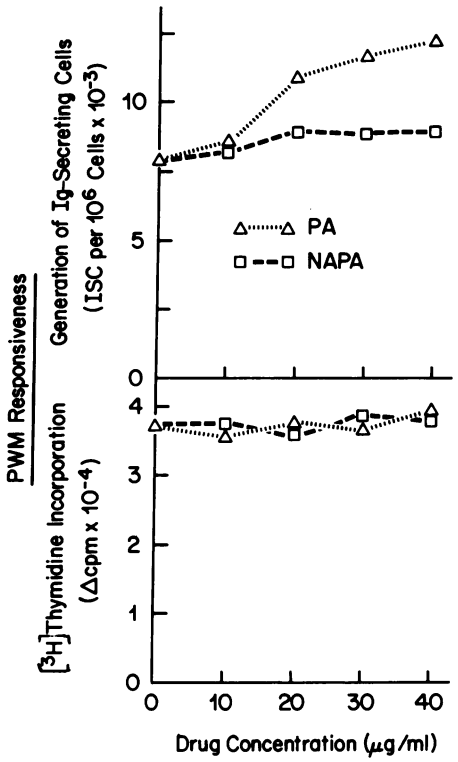

FIGURE 1 Effect of PA or NAPA on PWM-induced generation of ISC and [^{3}H]Tdr incorporation of PBM.

Effect of PA on T-cell regulation of mitogen-induced generation of ISC. To examine further the mechanism of PA-induced augumentation of the generation of ISC, experiments using purified T and B cells were done. B cells were supplemented either with control T cells or T cells that had been treated with mitomycin C. Since inhibition of T-cell proliferation by either irradiation or mitomycin C treatment has been shown to abbrogate suppressor cell function but permit the expression of helper cell function (17, 21, 22), this approach permitted an examination of the possibility that PA-mediated augmentation of ISC generation resulted from a selective effect on suppressor T-cell generation. Thus, it was reasoned that if PA were to augment ISC generation in cultures containing control T cells but not those containing mitomycin C-treated T cells, the effect could be attributed to interference with the generation of suppressor T cells.

TABLE I
Effect of PA on Mitogen Responsiveness

Mitogen	Response	Effect of PA* (Change in response)‡	P
		%	
PWM	ISC	+29±10	<0.02
	[^{3}H]Tdr incorporation	+3±5	NS
SPA	ISC	+13±6	NS
	[^{3}H]Tdr incorporation	−11±7	NS

* Concentration of PA, 30 μg/ml.

‡ Mean±SEM, n = 10. Percent change = (Response$_{PA}$/Response $_{control}$) × 100.

The results are shown in Fig. 2. When B cells were incubated with increasing numbers of T cells, PWM-stimulated generation of ISC increased as a function of the number of T cells added. At higher numbers of T cells ($>10^5$/microwell) suppression of the response was observed. This suppression with larger number of T cells was not observed when mitomycin C-treated T cells were used to support the cultures. When PA was added, the number of ISC generated was found to increase only in cultures supported by larger numbers of T cells (10^5 and 2×10^5/microwell). No effect of PA was observed when B-cell cultures were supported by smaller numbers of T cells, and no appreciable effect of PA was observed in cultures containing very large numbers of T cells (3×10^5/microwell). When B cells were co-cultured with mitomycin C-treated T cells, no significant effect of PA on the generation of ISC was seen. When B cells were incubated with either T cells or mitomycin C-treated T cells without PWM, no significant number of ISC was generated and no augmentation was seen as a result of the addition of PA. The absence of an effect in cultures containing mitomycin C-treated T cells indicates that PA inhibits suppressor T-cell activity. There appeared to be no augmentation of T-helper cell function or a direct effect on B-cell activity.

To determine whether NAPA also had an effect on T-suppressor cell function, similar experiments were carried out. As can be seen in Fig. 3, PA but not NAPA was found to have an inhibitory effect on T-suppressor cell activity.

When SPA was used as a mitogen, different results were obtained. SPA is a T cell-regulated polyclonal B-cell activator that is a more potent stimulator of suppressor T cells than PWM (17). As shown in Fig. 4, neither PA nor NAPA affected suppressor T-cell activity stimulated by SPA.

Effect of PA on the immunoglobulin (Ig) isotypes secreted in response to PWM. To determine whether the effect of PA on T-cell regulation is isotype specific, both IgG SC and IgM SC were individually enumerated in the cultures stimulated with PWM as described above. Results are shown in Fig. 5. Comparison of the number of ISC produced in cultures containing 1×10^5 T cells/microwell with those produced in cultures containing 3×10^5 T cells demonstrates significant T-cell suppression of the generation of both IgG SC and IgM SC, which is reversed by prior treatment of the T cells with mitomycin C. Following addition of PA to the cultures, both IgG SC and IgM SC increased significantly in the presence of 10^5 cells/microwell. On the other hand, when NAPA (30 μg/ml) was added to these cultures, there was no increase in either IgG SC or IgM SC. In the cultures containing mitomycin C-treated T cells, the addition of PA produced no augmentation of IgG SC or IgM SC. These results indicate that PA but not NAPA inhibits T-cell suppression of the generation of both IgG SC and IgM SC.

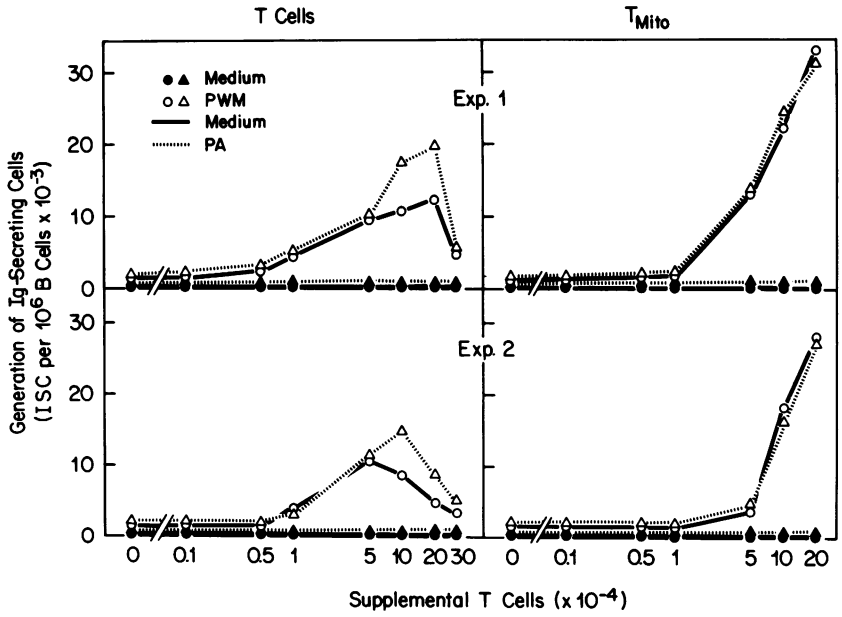

FIGURE 2 Effect of PA on T-cell regulation of the PWM response. B cells (2.5×10^4/well) were co-cultured with various number of T cells (left panel) or mitomycin C-treated T cells (right panel). PWM (10 μg/ml) or HBSS as a control was added. Cultures were carried out in the presence or absence of PA (30 μg/ml). T_{Mito}, mitomycin C-treated T cells.

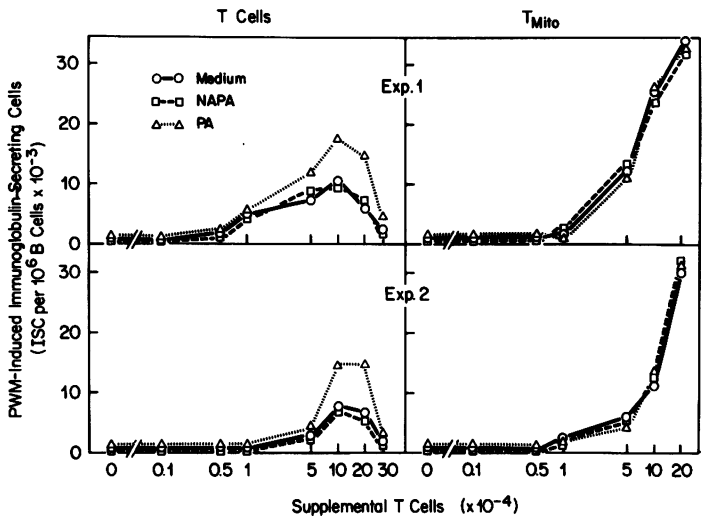

FIGURE 3 Effect of PA or NAPA on T-cell regulation of the PWM response. B cells (2.5 × 10^4/microwell) were co-cultured with various number of T cells (left panel) or mitomycin C-treated cells (right panel). As a stimulus, PWM (10 μg/ml) was used. PA (30 μg/ml), NAPA (30 μg/ml), or HBSS as a control was also added. T_{Mito}, mitomycin C-treated T cells.

Effect of PA on Con A-induced suppressor cell activity. To determine whether PA alters the generation or expression of suppressor T cells, experiments were carried out to examine the effect of this drug on the activity of suppressor cells induced by a preincubation with the mitogen, Con A. As shown in Fig. 6, PBM that had been preincubated with Con A were able to suppress markedly PWM responsiveness of fresh PBM. By contrast, control cells that had been preincubated with medium alone were not suppressive. Cells that had been preincubated with Con A in the presence of PA were much less suppressive than those preincubated with Con A alone. PA had no effect on control preincubated cells.

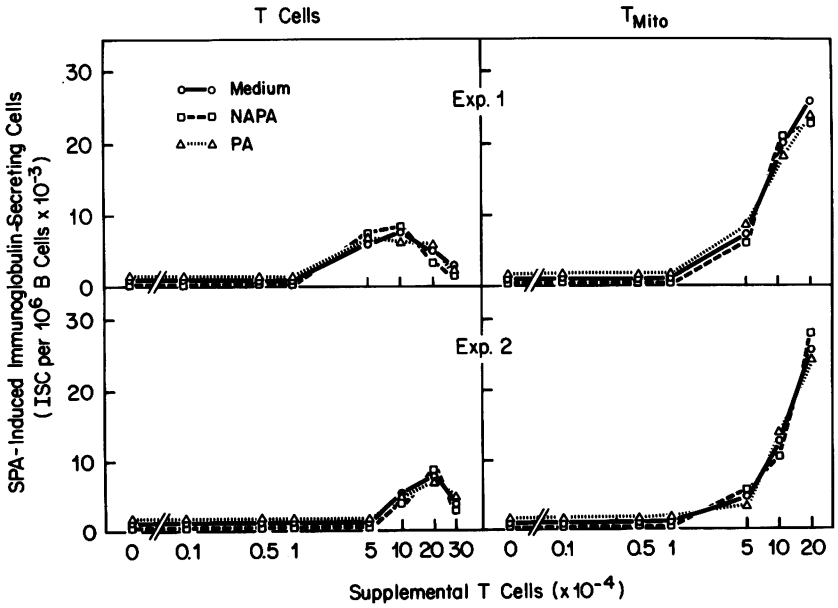

FIGURE 4 Effect of PA or NAPA on T-cell regulation of the SPA response. Experimental system was the same as in Fig. 3 except that SPA (1 μg/ml) was used as a stimulus. T_{Mito}, mitomycin C-treated T cells.

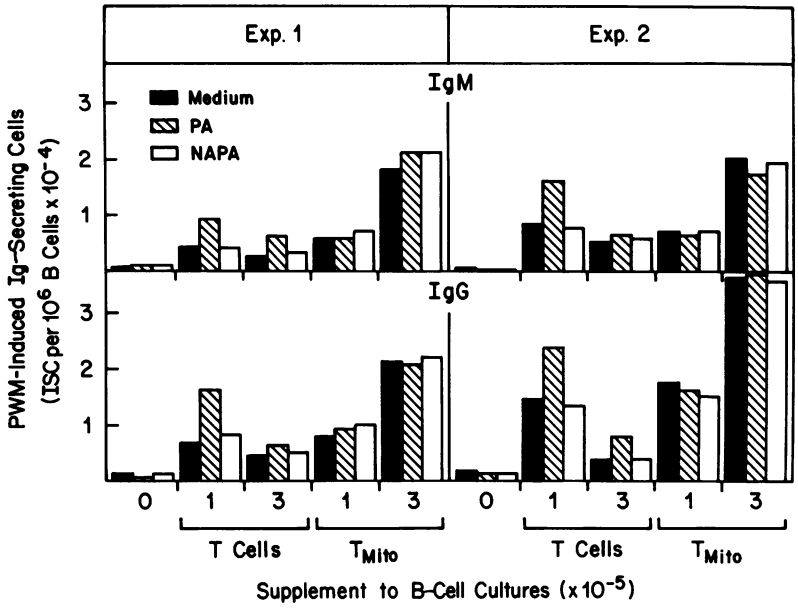

FIGURE 5 Effect of PA on PWM-stimulated ISC production. Experimental conditions were the same as in Fig. 3. IgG SC and IgM SC were enumerated using monospecific antisera.

The effect of various concentrations of PA on the generation of Con A-induced suppressor activity was examined in three individuals (Fig. 7). PA inhibited the generation of Con A-induced suppressor cells in a concentration-dependent manner with small but statistically significant inhibition observed with as little

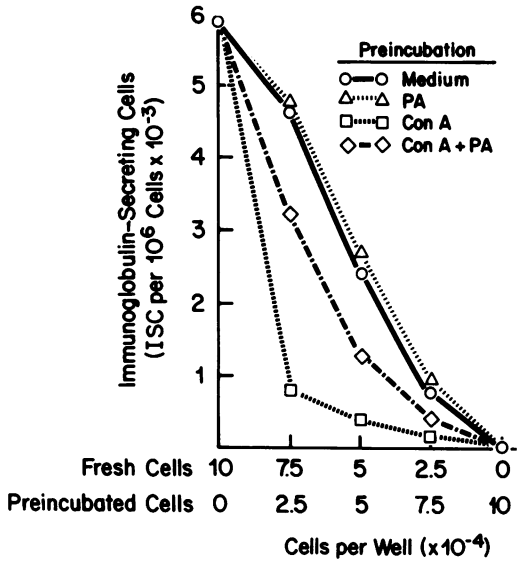

FIGURE 6 Effect of PA on the generation of suppressor cells by Con A. PBM were incubated with or without Con A (30 μg/ml) and with or without PA (30 μg/ml) for 48 h at 37°C. After the preincubation the cells were washed with α-methyl mannopyranoside and co-cultured with fresh PBM. After a 6-d incubation with PWM (10 μg/ml), the co-cultures were assayed for the number of ISC.

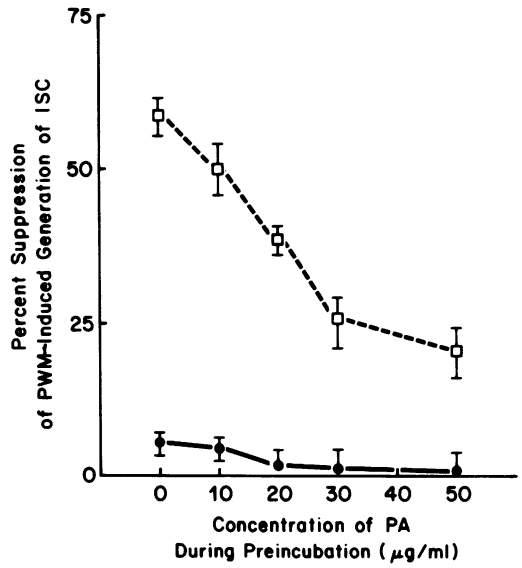

FIGURE 7 Dose-response effect of PA on the generation of suppressor cells by Con A. PBM were incubated with or without Con A (30 μg/ml) and with or without various concentrations of PA for 48 h at 37°C and then assayed for suppressor activity. Data represent the mean ±SEM for three experiments. Preincubation: medium (● —— ●); Con A (□- - -□).

as 10 μg/ml ($P < 0.01$, determined by repeated measures analysis of variance and Neuman-Keuls multiple comparisons).

Similar experiments were done using PBM from six different normal donors (Table II). In these experiments the number of ISC generated in co-cultures containing three parts of fresh responding cells and one part of the putative suppressor or control preincubated cells was determined. Either PA or NAPA was added during the culture used to generate the suppressor cells. Without Con A in the first culture, no suppressor cells were generated and PA was without effect. When Con A was present during the preincubation, suppressor cells were routinely generated. The mean percent inhibition of PWM responsiveness in these six experiments was 58.1%. When PA was included in the culture used to stimulate suppressor cells, the percent suppression decreased to 27.2 ($P < 0.005$, determined by one-way analysis of variance and Neuman-Keuls multiple comparisons). This decrease in suppression indicates that PA has an inhibitory action on the generation of Con A-induced suppressor function. This difference could not be explained by an alternation in the viability of the cells since the cells cultured with PA were >85% viable by trypan blue staining and not different in this respect from those cultured without PA. When NAPA was included in the culture used to generate suppressor cells, no effect on suppressor cell activity was observed. In three of six experiments, autologous PBM were used as target cells in the second cultures. There was no significant difference in the results obtained in the autologous or homologous co-cultures.

TABLE II
Effect of PA or NAPA on Con A-induced Suppression of ISC Generated in PWM-stimulated Cultures

Additions to cultures used to generate suppressor cells		Suppression of ISC
Con A*	Drug‡	Mean±SEM§
		%
0	0	1.5±3.5
	PA	−2.3±4.3
	NAPA	2.5±5.1
+	0	58.1±3.4‖
	PA	27.2±4.3‖
	NAPA	59.4±5.5

* Concentration of Con A, 30 μg/ml.
‡ Concentration of PA and NAPA, 30 μg/ml.
§ Values represent the mean percent decrease from predicted numbers of ISC in PWM-stimulated cultures. Six experiments are averaged. In three, the Con A-stimulated cells were homologous, in three they were autologous.
‖ Values differ significantly ($P < 0.005$).

TABLE III
Effect of PA on the Expression of Con A-induced Suppressor Activity

Preincubation to generate suppressor cells Con A*	Co-culture to assay suppressor cell function PA‡	Suppression of ISC§	
		Mean±SEM	Δ decrease
		%	
0	0	2.4±2.1	
0	+	−7.8±2.2	10.2
+	0	62.9±3.6	
+	+	47.7±2.8	15.2

* Concentration of Con A, 30 μg/ml.
‡ Concentration of PA, 30 μg/ml.
§ Values represent mean of 11 experiments. In eight, the Con A-stimulated cells were homologous, in three the cells were autologous.

The effect of PA on the second co-culture stimulated with PWM was examined. When PA was added only to the co-culture of fresh PBM (Table III) with Con A-activated suppressor cells, mean percent suppression of ISC decreased from 62.9 to 47.7% (Δ = 15.2%). On the other hand, when PA was added only to the co-culture of control preincubated cells with fresh PBM cells, the percent suppression of ISC decreased from 2.4 to −7.8% (Δ = 10.2%). Thus, the effect of adding PA only to the assay co-culture was similar in magnitude when either Con A-activated or control preincubated cells were present. These results suggest that the major action of PA when present in the assay co-culture does not relate to its action on the Con A-induced suppressor cells but rather to an additional effect on the generation of suppressor cells from the responding population. They support the conclusion that the major action of PA involves inhibition of the generation of suppressor cells and not the expression of suppressor cell function.

In some experiments, Con A-activated or control preincubated cells were treated with mitomycin C, co-cultured with fresh PBM and their action on Con A (10 μg/ml)-stimulated [^{3}H]Tdr incorporation examined. No significant effect of PA on the generation of Con A-induced suppression of [^{3}H]Tdr incorporation was observed in these experiments (data not shown).

DISCUSSION

Because PA induces autoantibody formation and SLE-like disease in a large proportion of treated individuals (7–10), experiments were carried out to determine whether this drug had an effect on the regulation of

the immune response. In this report we have shown that PA inhibits the generation of suppressor cells involved in regulating in vitro antibody formation. This was shown by experiments in which various numbers of control T cells or T cells treated with mitomycin C were added to a fixed number of B cells. The control T-cell population contained both suppressor and helper T-cell activities. However, the ratio of helper to suppression is normally such that suppressor activity is observed only when large numbers of T cells are used (17, 21, 22). When smaller numbers of T cells are used ($<5 \times 10^4$/culture) little or no suppressor function is apparent (17). In contrast, mitomycin C-treated T cells that cannot undergo cellular division are capable of generating only helper cell activity, regardless of the number of cells added to culture (17, 21, 22). Since the augmentation of ISC generation by PA was observed only in cultures containing large numbers of control T cells ($10–20 \times 10^4$/culture), it can be concluded that the effect of this drug is to impair the generation of suppressor T-cell activity. Since no augmentation of ISC generation was observed in cultures containing small numbers of control T cells nor with any number of mitomycin C-treated T cells, it can be concluded that PA exerted no enhancing effects on T helper cell, B cell, or macrophage activities. Furthermore, the use of the two-stage Con A pulse system for the generation of suppressor T cells confirmed the conclusion that PA altered suppressor cell function and also permitted the observation that PA exerted its major effect on the generation rather than the expression of suppressor cells. These in vitro findings support the idea that PA may affect the suppressor networks that normally regulate the production of antibodies in vivo. The dysfunction of such T-cell networks as a result of PA therapy could lead to the autoimmune reactions observed in patients treated with this drug.

PA did not exert a global effect on all suppressor systems tested but rather appeared to cause a selective inhibitory effect. For example, PA had no effect on the incorporation of [^{3}H]Tdr stimulated by PWM while significantly inhibiting PWM-stimulated T-cell suppressors of immunoglobulin secretion. Moreover, the addition of PA to Con A-stimulated cultures did not affect the ability of the suppressor cells generated to inhibit Con A-induced [^{3}H]Tdr incorporation of fresh cells. Finally, PA did not affect the generation of ISC by SPA and had no effect on SPA-induced suppressor cells. This would suggest that the effect of PA in altering immunoregulation may be selective. A selective effect would be consistent with the selective action this drug exerts in vivo as evidenced by the fact that some autoantibodies such as antihistone and anti-single stranded (ss) DNA antibodies are induced preferentially (3, 5).

Certain parallels can be observed between the in vitro experiments reported here and the in vivo situation observed in PA-treated patients. First, PA exerted its effect in vitro at concentrations as low as 10–20 μg/ml. Serum levels of treated patients are in this range (23), and sometimes higher (24), and tissue levels are generally higher (25). Second, PA augmented both IgM and IgG ISC, and autoantibodies of both classes have been observed in PA-treated individuals (6). A third parallel of special interest is that NAPA had no effect on suppressor function either of the PWM-generated or Con A-stimulated suppressor T-cell populations. This finding is consistent with the decreased ability of NAPA, compared with PA, to induce antinuclear antibody and anti-ssDNA antibody formation (26) and with its failure to exacerbate the lupus syndrome in patients in whom this condition had recently developed on PA (12).

It has been proposed that the autoimmune response in patients receiving PA is stimulated by an autoantigen formed in a reaction between PA, or metabolic products of PA, and an autologous macromolecule or membrane constituent (4, 11, 13). Such an interaction could result in a variety of autoantibodies including antinuclear antibodies specific for histones (5) and ssDNA (3, 27), as well as rheumatoid factor (1), antierythrocyte (1) and antilymphocyte (6) autoantibodies. However, the large number of autoantibodies evoked by PA has suggested, alternatively, that this drug acts directly on the immune system to stimulate autoantibody formation (6, 12, 14, 28). Bluestein et al. (6) observed a markedly increased frequency of cold-reactive lymphocytotoxic antibodies in patients treated with PA and a rapid disappearance of this antibody following withdrawal of PA in patients with PA-induced SLE. In addition, the lymphocyte response to phytohemagglutinin in vitro was reduced at elevated concentrations of PA and enhanced at therapeutic concentrations. They proposed that antinucleic acid antibodies might appear in PA-treated patients as a consequence of a disorder of immune regulation resulting from a reaction between PA and lymphocyte membranes. In addition, this same group has reported that when PA was added to antigen-driven cultures of sheep erythrocyte-primed rabbit spleen cells, a prolonged antibody-forming cell response was observed (29). This experiment suggested that PA acted by altering suppressor T-cell circuits that may regulate this in vitro response. The findings of our study are consistent with the hypothesis that PA interferes with normal immunoregulation through the mechanism of interfering with the generation of certain suppressor T-cell networks.

Genetic factors play a role in the autoimmune effect of PA. In patients receiving this drug, autoantibodies

occur more frequently and at higher titer, and PA-induced lupus develops more rapidly in subjects who are slow acetylators (11, 26). Moreover, an association of the development of drug-induced lupus with an HLA marker has been observed in the case of hydralazine. Batchelor et al. (30) have reported that almost all patients with hydralazine lupus were not only slow acetylators but that there also was an aggregation of the expression of the HLA-DR4 allele in this condition. Whether there is also an HLA association with PA-induced disease is not known.

Whether all drugs that have the capacity to induce the production of autoantibodies as well as SLE exert their effects by inhibition of autoregulatory T-cell function is at present unclear. Kirtland et al. (31) reported that methyldopa, which may induce the development of a variety of autoantibodies in treated patients (32), was capable of inhibiting suppressor T-cell function in vitro. This effect was observed in isolated T cells incubated with methyldopa and in T cells from patients taking methyldopa. Other studies demonstrating a selective effect on suppressor T-cell function by SLE-inducing drugs have not been reported, although it has been suggested that autoantibody formation induced by D-penicillamine therapy may also be related to suppressor T-cell dysfunction caused by this drug (33).

In conclusion, we have demonstrated that PA, but not its acetylated metabolite, is capable of interfering with the generation of suppressor T-cell activity in normal human peripheral blood lymphocytes following PWM and Con A stimulation. We propose that a similar immunomodulatory effect may be induced by PA in vivo leading to the production of autoantibodies in patients treated with drug (1, 2, 7, 10, 27).

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