Potentiation of Mitogen-induced Human T-Lymphocyte Activation by Retinoic Acid

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ABSTRACT

The capacity of retinoic acid to modulate human T-lymphocyte and B-lymphocyte activation by mitogens was examined. T-lymphocyte proliferation stimulated by 12-O-tetradecanoylphorbol-13-acetate (TPA) or phytohemagglutinin was enhanced by 5 nM to 5 μM retinoic acid in a dose-dependent manner with a 65 ± 35% (SD) increase (n = 6, P < 0.01) in TPA-stimulated proliferation induced by 5 μM retinoic acid. Retinoic acid enhanced T-lymphocyte proliferation over a wide range of background proliferation induced by different TPA concentrations. Retinoic acid alone did not stimulate T-lymphocyte proliferation. In contrast retinoic acid inhibited B-lymphocyte proliferation stimulated by TPA or phytohemagglutinin with 26.7 ± 23.4% inhibition of TPA-stimulated proliferation induced by 5 μM retinoic acid (P < 0.02). Retinoic acid had intermediate effects on the proliferation of different mixtures of T- and B-lymphocytes stimulated by TPA or phytohemagglutinin. The recognition that retinoic acid has opposing effects on human T- and B-lymphocyte activation by mitogens may account for the conflicting reports of the effects of retinoids on the immune response of unpurified human lymphocyte preparations.

INTRODUCTION

Vitamin A analogues collectively termed retinoids have attracted considerable interest as agents which may prevent the development of cancers (3, 17). The chemopreventative effect of retinoids in animal models of carcinogenesis may be attributed in part to the capacity of retinoids to potentiate immunity. Retinoid-treated mice have enhanced host-versus-graft reactivity (6, 10) and second-phase antibody responses (2) and demonstrate increased in vitro killer T-lymphocyte function (4) and cell-mediated cytotoxicity (9). However, the capacity of retinoids to potentiate immunity has not been reliably demonstrated using in vitro models of the human immune response. Retinoids have been reported to either inhibit (15) or enhance (16) human peripheral blood lymphocyte proliferation in response to mitogens. Retinoids may either augment (7) or diminish (1) natural killer activity. Retinoids also inhibit antibody-dependent cellular cytotoxicity (5). In contrast, retinoid acid enhances the proliferative response of human thymocytes and tonsillar lymphocytes to allogeneic stimulator cells but has no effect on the response of splenic and mixed peripheral blood lymphocytes to the same stimulus (14). In general these studies utilized mixed lymphocyte preparations which may account for the conflicting results. The capacity to purify human T- and B-lymphocytes now permits analysis of the effects of retinoids on purified lymphocyte subpopulations. This paper demonstrates that retinoic acid enhances mitogen-induced human T-lymphocyte activation and inhibits B-lymphocyte activation.

MATERIALS AND METHODS

Medium 199, RPMI-1640, sheep erythrocytes, and human AB-positive serum (Microbiological Associates Bioproducts, Walkersville, MD); macromolecular dextran-70 in 0.15 M NaHCO₃ saline (Macrodex; 6 g/100 ml); Ficoll:Hypaque (Pharmacia Fine Chemicals, Inc., Piscataway, NJ); 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, penicillin (100 IU/ml), and streptomycin (1000 μg/ml) (Gibco Laboratories, Grand Island, NY); PHA* (Difco Laboratories, Inc., Detroit, MI); and TPA and retinoic acid (Sigma Chemical Co., St. Louis, MO) were obtained as noted.

Isolation of Human T- and B-Lymphocytes. Lymphocytes from normal adult donors were obtained and processed under sterile conditions as described (11, 12, 18). Briefly after dextran sedimentation of the erythrocytes in heparinized blood, the mixed leukocytes in the plasma were sedimented by centrifugation, washed, and resuspended in M199-HPS. Ten-ml portions of 2 x 10⁶ leukocytes/ml were layered on 10-ml Ficoll:Hypaque cushions (sp gr, 1.076) and centrifuged at 4°C in order to achieve resetting of the T-lymphocytes. The mixture was then centrifuged at 100 x g for 5 min and placed in an ice-water bath for 60 min in order to achieve rosetting of the T-lymphocytes. The mixture was then centrifuged at 500 x g for 35 min at 37°C. The mixture was then centrifuged at 100 x g for 5 min and placed in an ice-water bath for 60 min in order to achieve rosetting of the T-lymphocytes. The mixture was then centrifuged at 500 x g for 35 min on Ficoll:Hypaque cushions at 4°C in order to separate the T-lymphocyte rosettes from less dense nonrosetting monocytes and B-lymphocytes. The later cell population was 40 to 50% B-lymphocytes. The T-lymphocyte-erythrocyte pellet was gently resuspended in 1 ml of human AB-positive serum, and 10 ml of sterile distilled H₂O were added for 15 s to lyse the sheep RBC by hypotonic exposure. The T-lymphocytes were quickly washed twice in M199-HPS and resuspended at a concentration of 1 x 10⁶/ml of M199-HPS with 15% (v/v) human AB serum. The purity of the T-lymphocyte preparation was assessed by rerosetting an aliquot of T-lymphocytes with sheep RBC and counting the number of rosettes. The purity was always greater than 97%, with fewer than 3% monocytes detected by a nonspecific stain for esterase activity (12). T-lymphocyte viability was greater than 97%, as determined by the exclusion of trypan blue dye.

Assessment of T-Lymphocyte Activation and Production of Prostaglandin E₂. The proliferative response of lymphocytes was measured by the incorporation of [³H]thymidine (11, 18). Two x 10⁵ purified T-lymphocytes in 0.2 ml of M199-HPS containing 15% (v/v) human AB-positive serum were added to each of quadruplicate wells of microtiter plates without or with the immunologically active agent to be studied and incubated at 37°C in 5% CO₂:95% air. After 72 h 1 μCi of [³H]-thymidine was added to each well and incubated for 16 h at 37°C. The radioactive supernatant was then removed and 400 μl of Insta-Gel scintillation fluid (New England Nuclear, Boston, MA) was added to each well. The radioactivity was counted using a Beckman LS-1000 liquid scintillation spectrometer.

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RESULTS

In initial studies of the modulation of TPA-stimulated T-lymphocyte proliferation by retinoic acid, T-lymphocytes were incubated for 72 h with a suboptimal concentration of TPA (5 ng/ml) without or with concentrations of retinoic acid between $5 \times 10^{-10}$ M and $5 \times 10^{-8}$ M. Proliferation was assessed by uptake of $[^3H]$thymidine which was added during the last 16 h of the incubation (Chart 1). In six experiments, retinoic acid increased the T-lymphocyte mitogenic response to TPA in a dose-dependent manner with a 65 ± 35% (SD) increase ($P < 0.01$, paired $t$ test) achieved with $5 \mu M$ retinoic acid. Incubation of T-lymphocytes with retinoic acid alone did not increase cellular proliferation, so the increased T-lymphocyte proliferation induced by retinoic acid reflects potentiation of TPA-stimulated mitogenesis. Concentrations of TPA between 2 and 10 ng/ml stimulated T-lymphocyte proliferation in a dose-related manner, and $5 \mu M$ retinoic acid significantly increased the proliferative response at all concentrations of TPA (Chart 2). In contrast 10 ng of the non-tumor-promoting phorbol esters 4α-phorbol and phorbol-12,13-diacetate per ml did not increase $[^3H]$thymidine uptake by T-lymphocytes above the 200 to 300 cpm uptake observed for untreated cells, and retinoic acid did not potentiate T-lymphocyte proliferation. Retinoic acid also potentiated T-lymphocyte mitogenesis stimulated by PHA (10 $\mu M$). Five $\mu M$ retinoic acid enhanced PHA-induced proliferation by 60 ± 34% (SD; $P < 0.01$, paired $t$ test) for six donors but inhibited proliferation by 38% for two donors. There was no apparent difference in the T-lymphocyte purity and monocyte content of the leukocyte preparations for the two groups of donors. However, PHA alone induced mean $[^3H]$thymidine uptake of 94,000 cpm for the retinoic acid-enhanced cells and 58,000 for the retinoic acid-inhibited cells.

The effect of retinoic acid on the mixed lymphocyte response was examined to determine if the potentiating effect of retinoic acid extended to other T-lymphocyte functions. In two experiments the proliferative response of T-lymphocytes to unrelated, mitomycin C-treated T-lymphocytes was increased by 27% when $5 \mu M$ retinoic acid was present throughout the incubation period. In the same experiments $5 \mu M$ retinoic acid increased TPA-stimulated T-lymphocyte proliferation by 32%, suggesting that retinoic acid has comparable potentiating effects on mitogen and mixed lymphocyte-induced proliferation.

These studies clearly demonstrated that retinoic acid enhances TPA-stimulated activation of purified human peripheral blood T-lymphocytes. Previous studies by other investigators which failed to demonstrate the capacity of retinoic acid to enhance human peripheral blood lymphocyte activation utilized less purified cell populations. Thus to gain a greater understanding of the cellular basis for these different results, the capacity of retinoic acid to enhance TPA- and PHA-induced proliferation of different peripheral blood mononuclear leukocyte populations was investigated (Table 1). The populations examined were mixed mononuclear leukocytes obtained from the first centrifugation of mixed peripheral blood leukocytes on Ficoll cushions, T-lymphocytes isolated by erythrocyte rosette formation and recentrifugation on Ficoll cushions, nonrosetting B-lymphocytes and macrophages isolated by recentrifugation on Ficoll cushions, and mixtures of the purified T-lymphocyte and B-lymphocyte: macrophage preparations. In three experiments $5 \mu M$ retinoic acid significantly increased TPA- and PHA-induced T-lymphocyte proliferation, whereas retinoic acid significantly inhibited B-lymphocyte proliferation induced by both mitogens. The effect of retinoic acid on the proliferation of the less purified cell populations was complex. Retinoic acid enhanced TPA-induced proliferation of mixed mononuclear leukocytes, whereas retinoic acid inhibited PHA-induced proliferation of mixed mononuclear leukocytes from two donors and enhanced proliferation of the mixed leukocytes from a third donor. The effects of retinoic acid on the
potential of T-lymphocyte activation by retinoids

Table 2
Modulation of T-lymphocyte production of prostaglandin E₂ by TPA and retinoic acid

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Production of prostaglandin E₂ (ng/5 x 10⁶ T-lymphocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>3.9 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TPA, 10 ng/ml</td>
<td>5.7 ± 2.5</td>
</tr>
<tr>
<td>Retinoic acid, 5 μM</td>
<td>3.5 ± 1.9</td>
</tr>
<tr>
<td>TPA, 10 ng/ml, + retinoic acid, 5 μM</td>
<td>4.3 ± 1.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SD (n = 3).

interaction of retinoic acid with contaminating cells, T-lymphocytes were purified further by incubating the cells in plastic dishes for 1 h at 37°C in 15% human AB serum to remove adherent macrophages. Macrophages comprised less than 1% of the cells in the enriched T-lymphocyte pools as assessed by nonspecific esterase staining. In two studies, retinoic acid increased TPA-stimulated lymphocyte proliferation by 117 and 43% for control T-lymphocytes and 65 and 117%, respectively, for macrophage-depleted T-lymphocytes. Similarly, macrophage depletion had no consistent effect on PHA-induced T-lymphocyte proliferation.

Recent studies have demonstrated that macrophages which contaminate T-lymphocyte preparations may produce substantial quantities of prostaglandin E₂, which is a potent inhibitor of T-lymphocyte proliferation (8). To determine if retinoic acid enhances T-lymphocyte proliferation by blocking prostaglandin production, aliquots of 5 x 10⁶ T-lymphocytes were incubated for 1 h at 37°C with buffer, TPA (5 ng/ml), 5 μM retinoic acid, or TPA (5 ng/ml) plus 5 μM retinoic acid. The PGE₂ released into the culture supernatants was then partially purified by chromatography on Sep Paks and quantitated by radioimmunoassay (Table 2). The mean production of PGE₂ was not significantly different for the T-lymphocytes. TPA increased PGE₂ production, and retinoic acid slightly suppressed this effect for T-lymphocytes from only 2 of 3 donors. In contrast, parallel studies of T-lymphocyte proliferation demonstrated that retinoic acid enhanced TPA-induced proliferation of T-lymphocytes from all three donors by 89 ± 27% (SD).

**DISCUSSION**

The current studies of the immunoregulatory capabilities of retinoic acid demonstrated that retinoic acid has distinct opposing effects on mitogen-induced proliferation of different lymphocyte classes. Concentrations of retinoic acid between 5 nm and 5 μM enhanced TPA-induced T-lymphocyte proliferation in a dose-related manner (Chart 1). This effect of retinoic acid is not dependent upon the background T-lymphocyte proliferation as enhancement of proliferation was observed over a wide range of TPA concentrations (Chart 2). In contrast to its effects on T-lymphocyte proliferation, retinoic acid suppressed TPA-induced B-lymphocyte proliferation and had intermediate effects on proliferation of mixtures of T- and B-lymphocytes (Table 1). The recognition that retinoic acid has opposing effects on T-lymphocyte and B-lymphocyte proliferation may account for the frequently contradictory reports of the effects of retinoic acid on human lymphocyte activation (7–12). These studies in general utilized partially purified lymphocyte preparations composed of uncharacterized mixtures of T-lymphocytes, B-lymphocytes, and recombined T-lymphocyte and B-lymphocyte:macrophage pools were intermediate between the effects of retinoic acid on the purified cell populations. To examine the possibility that enhanced T-lymphocyte proliferation by retinoic acid results from
macrophages. Different ratios of these cell types result in different effects of retinoic acid on lymphocyte proliferation (Table 1). Similarly undetected contamination of T-lymphocytes with other cells may account for the occasional study in which retinoic acid inhibits PHA-stimulated proliferation. That different lymphocyte classes have distinct responses to retinoic acid was suggested by the study of Siddell et al. (14) who demonstrated that retinoic acid enhanced human thymocyte proliferation but had no effect on peripheral blood lymphocyte proliferation. These authors did not, however, isolate peripheral blood T- and B-lymphocytes to examine this possibility further.

Enhanced T-lymphocyte activation by retinoic acid most likely reflects a direct effect of retinoic acid on T-lymphocytes, as highly purified cell populations were used. In contrast, B-lymphocyte preparations contained substantial numbers of macrophages, so the inhibitory effect of retinoic acid may reflect a macrophage-mediated effect rather than a direct effect of retinoic acid. Macrophages present in both T- and B-lymphocyte pools may enhance lymphocyte proliferation by the production of interleukins (13) or may inhibit proliferation by the production of arachidonic acid metabolites such as PGE2 (8,11). However, we and others (8) could not document significant changes in PGE2 production by highly purified T-lymphocytes stimulated with mitogen without or with retinoic acid (Table 2). Retinoic acid may alter the number and functional expression of lymphocyte cell surface receptors (5). Thus retinoic acid may modulate lymphocyte proliferation by altering expression of lymphocyte receptors for the mitogens TPA and PHA or lymphocyte receptors for endogenously produced mediators such as interleukins or arachidonic acid metabolites. Further investigations will be required to delineate the effects of retinoic acid on lymphocyte receptor expression.

REFERENCES

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