0022-202X/85/8405-0477$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 84:477–482, 1985

Vol. 84, No. 6
Printed in U.S.A.

Phosphodiesterase Inhibition by Ro 20-1724 Reduces Hyper-IgE Synthesis by Atopic Dermatitis Cells In Vitro

KEVIN D. COOPER, M.D., KEFEI KANG, M.D., SAI C. CHAN, B.S., M.S., AND JON M. HANIFIN, M.D.

Department of Dermatology, The Oregon Health Sciences University, Portland, Oregon, U.S.A.

Peripheral blood mononuclear leukocytes (MNL) from patients with atopic dermatitis spontaneously produce large amounts of IgE in vitro. These cells also show markedly elevated levels of cAMP phosphodiesterase (PDE) which may be responsible for the observed abnormal cAMP responsiveness. Treatment of atopic dermatitis MNL with varying concentrations of the cAMP PDE inhibitor Ro 20-1724 resulted in progressively decreasing amounts of IgE synthesis, statistically significant at the 10^{-4} M and 10^{-5} M concentrations. There was a close correlation between PDE inhibition and inhibition of IgE synthesis, $r = 0.93$, $p < 0.05$. To define the cellular target of the drug, we used monoclonal antibodies directed toward MNL subsets (Lyt 3, OKT8, OKT4, monocyte-myeloid) in a modified "panning" method to perform experiments with purified subsets. With untreated subsets, removal of OKT4-positive cells significantly reduced IgE synthesis; readdition of OKT4-positive cells enhanced IgE synthesis. OKT8 cells and monocytes did not affect IgE synthesis. Pretreatment of T cell-depleted MNL with Ro 20-1724 resulted in significantly more inhibition of IgE synthesis than did pretreatment of T enriched cells prior to recombination with the reciprocal untreated subset and subsequent culture. Similarly, pretreatment of monocyte-depleted cells resulted in significantly more inhibition of IgE synthesis than pretreatment of monocyte-enriched cells prior to recombination and culture. The majority of the effect appeared to be mediated by a direct effect on the B cells. However, some inhibition of IgE synthesis was also achieved through pretreatment of T enriched cells. Since pretreatment of isolated suppressor/cytotoxic or helper/inducer T-cell subsets did not give the same degree of inhibition as with unfractionated T cells, a T–T interaction may be involved in this aspect.

The imidazolidinone derivative, Ro 20-1724, significantly and consistently inhibited both the elevated cAMP phophodiesterase activity and the elevated spontaneous IgE synthesis of MNL from patients with atopic dermatitis. These findings demonstrate a previously undescribed link between cAMP PDE levels and in vitro IgE synthesis.

Manuscript received June 4, 1984; accepted for publication November 28, 1984.

Supported in part by NIH Training Grant 2 T32 AM07153 and NIH Grant 1 T01 AI18615 and by a grant from the Medical Research Foundation of Oregon.

Reprint requests to: Kevin D. Cooper, M.D., Dermatology Branch, National Institutes of Health, Bethesda, Maryland 20205.

Abbreviations:
AD: atopic dermatitis
BSA: bovine serum albumin
cAMP: cyclic AMP
FCS: fetal calf serum
MNL: mononuclear leukocyte(s)
Mo: monocyte
PBS: phosphate-buffered saline
PDE: phosphodiesterase
PGE_1: prostaglandin E_1
Ro: Ro 20-1724

Patients with atopic dermatitis (AD), a disease characterized by high tissue histamine levels [1,2], demonstrate a variety of immunologic and pharmacologic abnormalities. We have shown that blood leukocytes from patients with AD show markedly reduced cAMP responses to isoproterenol, histamine, and prostaglandin E_1 (PGE_1) [3]. Similarly reduced responses were observed in normal leukocytes desensitized by micromolar concentrations of histamine [3,4].

It appears that this lack of cAMP responsiveness is due to rapid hydrolysis of cAMP by increased intracellular cAMP phosphodiesterase (PDE) as evidenced by our findings that cells from patients with AD, as well as normal cells exposed to low concentrations of histamine, have a dramatic and consistent elevation of a high-activity form of PDE [4,5]. Increased leukocyte cAMP PDE and consequent lack of cAMP-mediated immunoregulatory influences may provide a permissive effect on leukocyte functions such as IgE production [6] and histamine release [7].

Patients with AD have elevated serum IgE levels [6] and this is reflected in the finding that AD leukocytes placed in culture spontaneously produce elevated levels of IgE over a 7- to 10-day culture period [8–11]. While largely independent of radioresistant T-cell help [9,11], the IgE-producing B cells are sensitive to suppression by high numbers of added allogeneic normal T cells. Although purely allogeneic effects were not excluded in all these studies [8,12,13], others have excluded this mechanism [9]. Several of these studies revealed a lack of T suppressor activity by cells from AD or hyperimmunoglobulinemia E syndrome patients but these findings have not been consistent, and some patients, whose mononuclear leukocytes (MNL) overproduce IgE, clearly have normal T suppressor function [8,9,11,12]. A recent study showed that T-cell supernatants from patients with AD enhanced IgE synthesis by B cells [14]. The complete role of T lymphocytes in elevated IgE production by cells from patients with hyperimmunoglobulinemia E states remains uncertain.

The purpose of this study was to determine the effect upon in vitro IgE synthesis by leukocytes from patients with AD when the elevated PDE activity was lowered by the PDE inhibitor Ro 20-1724 (d,l-1,4-[3-butoxy-4-methoxybenzyl]-2-imidazolidinone). Using a modification of the "panning" method for sorting leukocytes with monoclonal antibodies [15–19], the leukocyte subsets affected by the drug were identified.

MATERIALS AND METHODS

Experimental Subjects

Normal, nonatopic adult volunteers and adult atopic patients, ages 18–50 years, were phlebotomized between 8:00 and 10:00 AM after overnight avoidance of coffee and other PDE inhibitor-containing beverages. Therapeutic agents such as antihistamines and topical steroids were discontinued at least 48 h prior to the study. Systemic corticosteroids and theophylline were discontinued at least 2 weeks prior to study. All patients had asthma and/or allergic rhinitis in addition to AD.

In Vitro IgE Production

To study the in vitro effect of Ro 20-1724 on IgE production, MNL were separated from heparinized venous blood by Ficoll-Hypaque gradient centrifugation, washed 3 times in saline, and resuspended in

RPMI 1640 (Gibco, Grand Island, New York). For spontaneous IgE production in vitro, cells were adjusted to 1.5×10^6/ml in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, penicillin 100 U/ml, streptomycin 100 μg/ml, and amphotericin B 0.25 mg/ml. The cell suspension (total volume, 1.5 ml) was incubated in 12 × 75 mm polystyrene tubes (Falcon 2054) in a 5% CO_2 humidified atmosphere at 37°C for 10 days. The cells were subjected to 5 cycles of freeze-thaw and held in culture for 3 more days to allow microsomal release of all immunoreactive IgE. Culture supernatants were harvested on day 13 by centrifugation at 500 *g* for 10 min and stored at −20°C until assayed. To determine preformed IgE, controls were incubated with cycloheximide 500 g/ml for 10 days or subjected to freezing and thawing 5 times on day 0 [8]. Preformed IgE levels did not significantly differ between these methods. (Cycloheximide method: 606 ± 285 pg/ml preformed IgE vs freeze and thaw method: 767 ± 468 pg/ml, n = 5 paired comparisons.)

For determination of the Ro 20-1724 dose response on IgE production in vitro, cells were adjusted to 1.5×10^6/ml in 1.5 ml RPMI 1640 supplemented with 2 mM glutamine and antibiotics as mentioned above (2.25×10^6 total cells/culture). A stock solution of Ro-20-1724 was prepared and frozen in Ro 20-1724. The solution was sterilized by filtration through 0.45-μm filters. The cell suspensions were incubated with or without Ro 20-1724 (provided by Hoffman-LaRoche, Nutley, New Jersey) at final concentrations of 10^{-6} M, 10^{-5} M, 10^{-4} M in serum-free RPMI for 1 h at 37°C in a humidified 5% CO_2–95% air incubator. After incubation, the cells were washed and cell suspensions were reconstituted with RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, and antibiotics and incubated at 37°C as described above.

Separation of Subpopulations

In initial experiments, unfractionated MNL were used. However, in further experiments, we used a modified "panning" method to fractionate MNL subsets [15–19]. These populations were sorted into subset-depleted and -enriched populations by a modification of the panning method. The major modification was the sorting of subsets from unfractionated MNL rather than from E rosette-purified T cells. This allowed direct comparisons of IgE synthesis by marker-depleted MNL while retaining similar purity to that achieved with prior E rosetting [19]. Purified mouse monoclonal antibody (Lyt 3, New England Nuclear), against the human sheep erythrocyte receptor antigen, was used to purify Lyt 3-positive and Lyt 3-negative leukocytes. Similarly, OKT_4 helper/inducer antibody and OKT_8 suppressor/cytotoxic antibody (Ortho Diagnostics) and an antimonocyte (Mo) antibody (provided by Dr. M. Kamoun) were used to separate T-cell subsets and monocytes. For controls, MNL were sensitized with mouse ascites fluid (Bethesda Research Labs, Bethesda, Maryland) in place of monoclonal antibody. In brief, MNL were sensitized with the appropriate dilution of antibody for 30 min, washed, and added to a polystyrene Petri dish coated with goat antimouse IgE. The plates were incubated at 4°C for 40 min, swirled, and incubated for another 30 min. Marker-depleted cells were harvested as the nonadherent fraction and the marker-enriched cells were harvested from the adherent fraction by vigorous pipetting. The nonadherent, marker-negative cells were then added to a freshly coated plate for better purity. Marker-negative cells had fewer than 5% positive cells (i.e., greater than 95% pure) whereas T cell marker-positive cells were greater than 85% marker positive (i.e., 85% pure) as determined by fluoresceinated goat antimouse IgG staining. Fewer than 2% B cells were present in the positively selected populations. Monocyte-depleted cells were 88% pure and monocyte-enriched cells were 67% pure as determined by latex ingestion. Ro 20-1724 at varying concentrations of medium alone was then added to either the marker-negative or marker-positive population for 1 h at 37°C in a humidified 5% CO_2 atmosphere. The cells were washed and the treated subpopulation combined with the untreated reciprocal population and cultured for 10 days. The cell subset number per culture was that derived from 2.5×10^6 MNL sensitized with monoclonal antibody prior to panning. After 10 days, the cells were freeze-thawed 5 times, cultured 3 more days, spun at 500 *g* for 10 min, and the supernatant frozen for IgE determination. Purity of the cell subpopulations was monitored by indirect immunofluorescence, latex ingestion, and surface immunoglobulin determination as previously described [20].

IgE Radioimmunoassay

The quantitative radioimmunoassay for IgE was performed using polyvinyl 96-well microtiter plates [9]. For measurement of IgE production in vitro, microtiter plate wells were filled with 300 μl 1:100 goat antihuman IgE (Meloy, Springfield, Virginia). After incubation in a humidified chamber at 23°C overnight, the coating antibody solution was removed and saved for reuse. The wells were washed individually 3 times with 1% bovine serum albumin (BSA) in 0.01 M phosphate-buffered saline (PBS), pH 7.4, and 5% BSA in PBS was added to the wells for 1 h. After another wash with 4% BSA in PBS, 250 μl of samples to be assayed were added to wells and incubated overnight in a humidified chamber at 23°C. The following day the samples were removed and discarded. After 3 washes with 1% BSA in PBS, 250 μl of monospecific affinity-purified ^{125}I-labeled goat antihuman IgE (Tago, Burlingame, California) was added to each well and incubated overnight in a humidified chamber at 23°C. The following day the plates were washed 3 times with 1% BSA in PBS and 7 times in running tap water. The individual wells were cut apart and the bound radioactivity was determined on a Beckman Gamma 4000 Counter (Irvine, California). For each radioimmunoassay, a 9-point standard curve of IgE (Phadebas Reference Serum, Pharmacia, Uppsala, Sweden) was performed in parallel with the culture samples.

For preparing ^{125}I-labeled goat antihuman IgE, 0.2 ml goat antihuman IgE was added into 0.5 ml PBS and concentrated to 0.10–0.15 ml in a collodian bag (Schleicher and Schuell, Keene, New Hampshire) through vacuum aspiration. Concentrated antibody was removed to a capped vial (4 ml) and then 10 μl chloramine T (10 mg/ml) and 5 μl ^{125}I-labeled solution (1 mCi/10 μl, New England Nuclear) were added and fully mixed with a metal stirring rod on a magnetic stirrer. Ten minutes later, 12 μl sodium metabisulfite (10 mg/ml) in phosphate buffer was added to stop the reaction. Non-protein bound iodide was removed by dialysis in the cold against 0.01 M PBS, pH 7.4, with 2 changes over a 16- to 20-h period. Labeling was performed fresh once weekly.

Phosphodiesterase Assay

Cells 10^7/ml in Gey's balanced salt solution were homogenized using a Polytron PP 10-32 at 4°C and assayed using modification of the procedure of Thompson et al [21]. The incubation mixture (0.4 ml) contained 0.02–250 M cAMP, 20,000 cpm [^{3}H]cAMP, and 0.2-ml sample in 40 mM Tris-HCl buffer (pH 8.0) containing 3.75 mM beta-mercaptoethanol and 50 mM $MgCl_2$. After incubation (30°C for 10 min) the reaction was terminated by snap-freezing in ethanol/dry ice and the mixture was boiled for 1 min. Purified 5′-nucleotidase (0.45 units) was added to mixtures which were incubated at 30°C for 10 min, then transferred to Pasteur pipette columns containing AG1X2 resin and counted in Aquasol 2. Enzyme activity was expressed as pmol cAMP hydrolyzed per min/10^6 cells. Background was less than 5%.

RESULTS

Unfractionated MNL Spontaneous In Vitro IgE Production

Table I demonstrates preformed IgE from MNL from patients with AD freeze-thawed on day 0 and harvested on day 3 compared with spontaneously produced total IgE from cells freeze-thawed on day 10 and harvested on day 13. The third column gives the newly synthesized IgE in these cultures (total IgE minus preformed IgE) over 10 days. Variation among individuals ranged from 562–5419 pg/ml. The correlation of serum IgE with newly synthesized IgE was +0.67 ($p < 0.05$).

TABLE I. *Preformed and newly synthesized spontaneous in vitro IgE production*[a]

Preformed[b]	Total[c]	Newly synthesized[d]
941	2510	1569
1125	2078	953
50	2482	2432
1681	5555	3874
50	5469	5419
190	1032	842
2662	4014	1352
305	3354	3049
721	5228	4507
135	697	562
222	2292	2070

[a] Expressed as pg/ml IgE.
[b] Freeze-thaw day 0, harvest day 3.
[c] Freeze thaw day 10, harvest day 13.
[d] Total-preformed.

For comparison, all 10 normal individuals studied produced less than 500 pg IgE/ml. We noted a positive correlation of newly synthesized IgE in vitro with serum IgE ($r = +0.67$).

Inhibition of In Vitro IgE Synthesis by Ro 20-1724

Fig 1 shows the effect of treatment with varying concentrations of the PDE inhibitor Ro 20-1724 on newly synthesized spontaneous in vitro IgE production. Progressively decreasing amounts of IgE were produced with increasing concentrations of Ro 20-1724. At 10^{-6} M, 1520 ± 443 pg/ml were produced; at 10^{-5} M, 1317 ± 282 pg/ml were produced; at 10^{-4} M, 917 ± 198 pg/ml were produced. There was a statistically significant decrease in IgE synthesis by the 10^{-5} M-treated MNL and the 10^{-4} M-treated MNL, $p < 0.05$, n = 10 patients. IgE synthesis was inhibited in 9 out of 10 of these patients and 10/10 other patients in whom preformed IgE was not determined (not shown).

We determined the correlation of inhibition of IgE synthesis to the degree of inhibition of PDE activity achieved by the inhibitor Ro 20-1724. On day 10, the MNL cell cultures from 6 patients were centrifuged, the pellets harvested for intracellular PDE determination, and the supernatants analyzed for in vitro IgE production. Fig 2 demonstrates a close relationship between the degree of PDE inhibition and inhibition of IgE production, with a correlation coefficient of 0.93, $p < 0.05$.

The viability of cells incubated with Ro 20-1724 was compared with untreated cells. Trypan blue exclusion showed no significant cell loss at any of the Ro 20-1724 concentrations.

Effect of Removal and Readdition of Untreated MNL Subsets on IgE Synthesis

In preliminary experiments for the determination of the leukocyte target of Ro 20-1724 we explored the interaction of T cells, suppressor/cytotoxic T cells, helper/inducer T cells, and monocytes with the IgE producing B cells.

Fig 3 shows IgE synthesis in pg/ml by unfractionated control MNL, MNL depleted of leukocyte subsets by panning and subset marker-depleted MNL recombined with the reciprocal marker-enriched subset. IgE synthesized by MNL sensitized with mouse ascites fluid and subjected to the panning procedure was not statistically different from control MNL ($p > .05$, n = 6). Removal by panning of OKT8-sensitized and antimonocyte antibody-sensitized MNL did not result in a significant alteration of IgE synthesis. By contrast, Lyt 3 (pan T)-depleted MNL showed somewhat reduced IgE synthesis as compared to controls (n = 9 pairs) ($p < 0.10$). More specifically, depletion of OKT4$^+$ helper/inducer T cells resulted in reduced IgE synthesis as compared to controls. Recombinance of Lyt 3$^+$ cells or OKT4$^+$ cells with the subset-depleted MNL restored IgE synthesis to levels not statistically significant from controls. Thus, spontaneous in vitro IgE synthesis by AD B cells was not suppressible by autologous OKT8-positive cells or monocytes, but was partially dependent upon helper effects of the OKT4$^+$ subset.

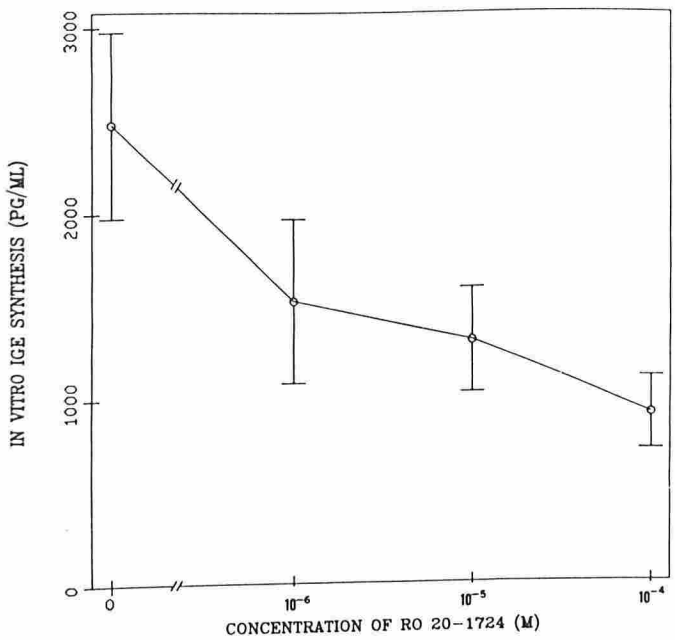

FIG 1. Effect of Ro 20-1724 on spontaneous in vitro IgE synthesis by MNL from 10 patients with atopic dermatitis. Inhibition of IgE synthesis at 3 concentrations of Ro 20-1724, expressed as newly synthesized IgE in pg/ml.

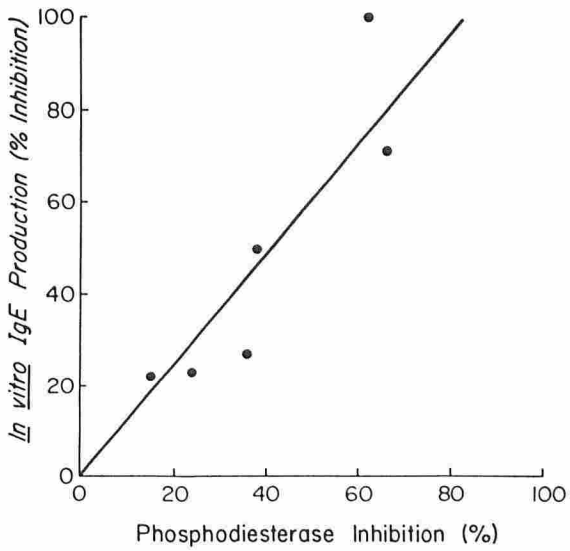

FIG 2. Correlation of inhibition of IgE synthesis with the degree of cAMP PDE inhibition induced by 10^{-4} M Ro 20-1724. Correlation coefficient = 0.93, $p < 0.05$; n = 6 patients.

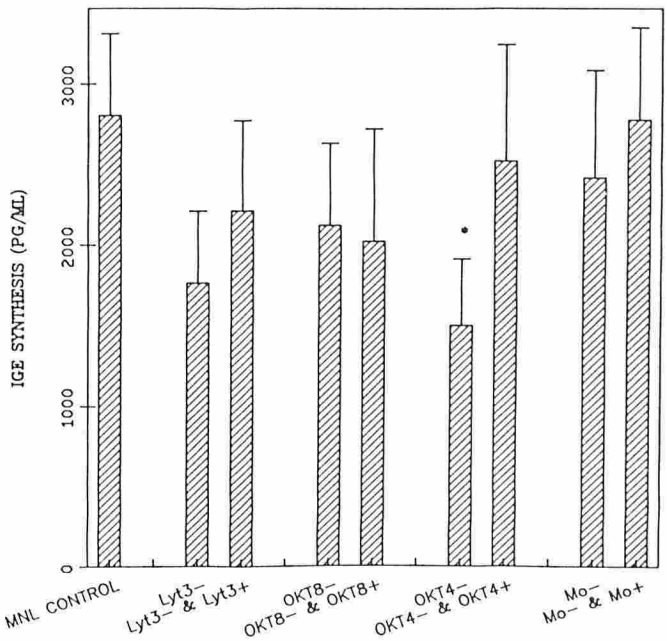

FIG 3. Effect of untreated subset depletion and recombination on spontaneous in vitro IgE synthesis. *Bars* are denoted as marker depleted MNL (e.g., Lyt 3$^-$) or marker-depleted MNL recombined with marker-enriched cells (e.g., Lyt 3$^-$ and Lyt 3$^+$). The *asterisk* indicates significant reduction in IgE synthesis: $p < 0.01$, n = 7.

Ro 20-1724 Pretreatment of Marker-Enriched and Marker-Depleted Cells

To determine the cellular target of the drug, subsets of MNL purified by panning were incubated at 37°C for 1 h in a humidified CO_2 incubator with or without 10^{-5} M or 10^{-4} M Ro 20-1724 in serum-free RPMI.

The treated subsets were washed 3 times prior to recombination with the reciprocal subset and culture for IgE production. Results were expressed as % change from untreated identical subset recombinations.

Using the Lyt 3 pan T-cell antibody, Lyt 3^+ (T-enriched) and Lyt 3^- (T-depleted) cells were prepared. As seen in Fig 4, although pretreatment of Lyt 3^+ cells resulted in some inhibition of IgE synthesis by untreated Lyt 3^- cells, pretreatment of Lyt 3^- cells resulted in significantly more inhibition of IgE synthesis. At 10^{-5} M Ro 20-1724, pretreatment of Lyt 3^+ cells resulted in a 16 ± 2% decrease vs a 39 ± 16% decrease when Lyt 3^- cells were pretreated, n = 8 pairs, $p < 0.05$. At 10^{-4} M, pretreatment of Lyt 3^+ cells resulted in a 25 ± 11.4% decrease vs a 52 ± 10.3% decrease when Lyt 3^- cells were pretreated, n = 8, $p < 0.05$. Thus, cAMP PDE pretreatment of the Lyt 3^- population, composed primarily of B cells and monocytes, resulted in significantly more inhibition of IgE synthesis than did pretreatment of the T cell-enriched Lyt 3^+ population.

To investigate whether Ro 20-1724 inhibition of IgE synthesis by the Lyt 3^-, B cell–monocyte mixture was due to activation of monocyte suppressive influences, a monoclonal antibody specific for a determinant found on human peripheral blood monocyte and myeloid cells was used to purify adherent monocyte-enriched and nonadherent monocyte-depleted cells. There was no effect (in % change from untreated control cells) of 10^{-4} M Ro 20-1724 pretreatment of purified monocytes prior to recombination with untreated monocyte-depleted cells (7 ± 13% increase). In contrast, monocyte-depleted cell pretreatment resulted in marked inhibition of IgE synthesis (71 ± 13% decrease, $p < 0.01$, n = 6) of similar degree as that seen with T depleted cells (48 ± 32% decrease) and unfractionated MNL (54 ± 8% decrease). Thus, Ro 20-1724 did not inhibit IgE synthesis through stimulation of suppressive monocytes and the full effect was achieved in the absence of monocytes, presumably through a direct effect on B cells.

Since IgE synthesis was partially dependent on $OKT4^+$ cells (Fig 3) and a 29% decrease in IgE synthesis was observed when Lyt 3^+ cells were pretreated with Ro 20-1724 (Fig 4), we examined whether the degree of inhibition seen with Lyt 3^+ pretreated cells was statistically significant. In order to use the untreated control cells as an experimental group to compare to the pretreated cells, the data were expressed as % change from untreated MNL IgE synthesis. Untreated Lyt 3^+ cells recombined with Lyt 3-depleted MNL showed a minimal 17 ± 14% decrease in IgE synthesis, whereas 10^{-4} Ro 20-1724-pretreated Lyt 3^+ cells recombined with Lyt 3^- showed a 47 ± 11% decrease in IgE synthesis, $p < 0.001$, n = 6. Thus, although not as effective as Lyt 3-depleted MNL, Lyt 3^+ cells also mediate inhibition of IgE synthesis when exposed to Ro 20-1724.

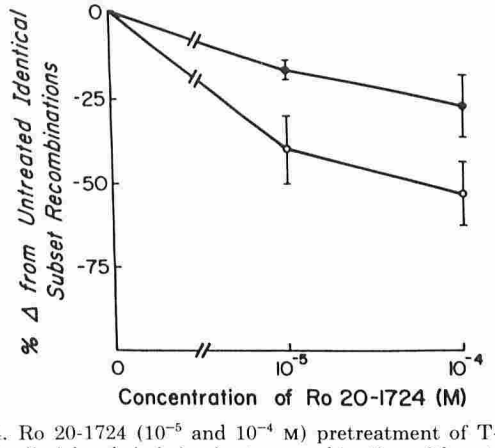

FIG 4. Ro 20-1724 (10^{-5} and 10^{-4} M) pretreatment of T-enriched (Lyt 3^+) cells (*closed circles*) prior to recombination with untreated T-depleted (Lyt 3^-) cells and in vitro culture for spontaneous IgE synthesis as compared to pretreatment of T-depleted (Lyt 3^-) cells (*open circles*) prior to recombination and culture. Results are expressed as % change in IgE synthesis from the untreated identical subset recombination (Lyt 3^+ plus Lyt 3^-). n = 6.

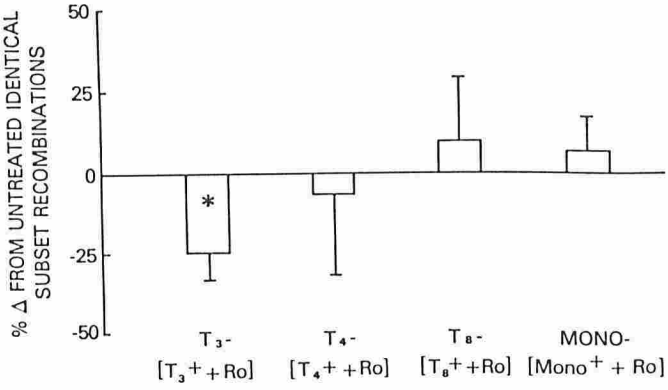

FIG 5. Ro 20-1724 (10^{-4} M) pretreatment of positively selected subsets [Lyt 3^+, $OKT4^+$, $OKT8^+$, antimonocyte$^+$ (Mo^+)] prior to recombination with the reciprocal subset-depleted MNL and in vitro culture for spontaneous IgE synthesis. n = 6. Only pretreatment of Lyt 3^+ (T-enriched) cells resulted in a significant change in IgE synthesis (*asterisk*).

We further examined the above T cell-mediated inhibition of IgE synthesis to determine whether Ro 20-1724 altered helper and suppressor T-cell function in this system. Fig 5 shows the effect of 10^{-4} M Ro 20-1724 pretreatment of positively selected subsets prior to recombination with the reciprocal IgE-producing negative subsets (i.e., Lyt 3^-, $T4^-$, and $T8^-$). The left bar shows the statistically significant inhibition previously demonstrated with pretreatment of $T3^+$ cells. However, inhibition was not seen when either $T4^+$ helper T cells or $T8^+$ suppressor T cells (n = 6) were pretreated with Ro 20-1724. The similar lack of effect with monocytes is shown for comparison. These data suggested that, in order to generate T-cell inhibition of IgE synthesis by Ro 20-1724, both $T4^+$ and $T8^+$ cells must be present together during Ro 20-1724 exposure.

DISCUSSION

These investigations have focused on a possible point of convergence between cyclic nucleotide regulatory and immunologic regulatory defects in AD. The cyclic nucleotide regulatory defects at present seem to center on the marked elevations of cAMP PDE activity in MNL from patients with AD [5]. This elevation is most likely responsible for the reduced cAMP responsiveness of these cells to all cAMP agonists tested [3,4]. The most obvious and consistent immunologic regulatory defects in atopy are the hyperimmunoglobulinemia E [6] and the elevated spontaneous in vitro IgE synthesis by B cells from patients with AD [8,13]. The central finding reported here, that reduction of cAMP PDE with Ro 20-1724 in AD MNL results in normalization of IgE production by AD MNL, suggests that cyclic nucleotide and immunologic defects in AD are linked events.

Ro 20-1724 is an imidazolidinone derivative with inhibitory action specific for cAMP PDE. We have found the drug to be 10- to 100-fold more potent than theophylline and 10-fold less potent than isobutylmethylxanthine in our system. In the same study, we showed that the high activity PDE in MNL from patients with AD is much more sensitive to PDE inhibitors than PDE from normals [22].

We have previously provided evidence that elevated cAMP PDE in AD resulted in reduced cAMP levels in response to all

cAMP elevating agents tested (histamine, catecholamines, and PGE_1) [3,5]. As shown by Goldstein and coworkers, the thymic hormone thymopoietin mediates its inductive effect on T-cell differentiation through cAMP elevation [23,24]. Abnormally differentiated T cells may be involved in elevated IgE production in AD. Similarly, thymic hormones have inhibitory influences on early B-cell differetiation [24–26], which may also be cyclic nucleotide mediated [24]. Thus, differentiative signals, normally mediated through cAMP, which promote T-cell differentiation or inhibit B-cell differentiation would not be delivered to the interior of the cell in AD. Correction of the abnormal PDE in AD cells may have allowed normalization of cAMP responsiveness to regulatory influences by other cells with resultant inhibition of spontaneous IgE synthesis.

Our prior findings of reduced numbers of beta-adrenergic receptors, reduced cAMP responses, and extremely high PDE levels in purified B cells suggested that B cells are normally protected from cAMP elevations and that they might be particularly sensitive to direct inhibition by PDE inhibitors [27].

We therefore performed experiments to determine which MNL subpopulations were mediating the inhibition of IgE synthesis induced by Ro 20-1724. There was significantly more inhibition when T cell-depleted MNL (B cell- and monocyte-enriched) were pretreated with Ro 20-1724 than when T-enriched cells were pretreated (Fig 4). We examined whether the effect on T cell-depleted MNL cells was mediated through activation of suppressive monocytes or directly through B cells. Monocyte-depleted MNL cells pretreated with Ro 20-1724 also were significantly more inhibited than pretreated monocyte-enriched cells. Thus, the majority of the effect was mediated through a non-T, nonmonocyte target, presumably through a direct effect on the IgE producing B cells. An effect on null cells is a remote possibility. The lack of inhibition seen with pretreated monocytes also rules against the possibility that inhibition seen with pretreated T cells was due to carryover of low concentrations of Ro 20-1724 to the sensitive B cells in the Lyt 3^- population.

This type of direct intervention with B-cell function could be expected based on the preactivated, terminally differentiated nature of the spontaneous IgE producing B cells involved [8,9,11], and on the high levels of cAMP PDE which B cells maintain [27]. Similar direct inhibition of antigen-specific human B cell IgE production has been achieved through antigen-induced unresponsiveness of the specific IgE producing B cells [13,28].

We also observed a significant degree of inhibition when Lyt 3^+ cells were pretreated with Ro 20-1724. Since in vitro IgE synthesis was partially dependent on $OKT4^+$ helper cell influences (Fig 3) [14], but unregulated by $OKT8^+$ suppressor cells (Fig 3), inhibition of these $OKT4^+$ helper cell influences by Ro 20-1724 seemed most likely. When purified $OKT4^+$ and $OKT8^+$ subsets were pretreated, no statistically significant changes in IgE synthesis were seen (Fig 5), although the trend tends to support the above view. Full T-cell inhibition may require both the OKT4 helper and the OKT8 suppressor/cytotoxic cells to be present during the Ro 20-1724 pretreatment. This suggests that some interaction between OKT4 and OKT8 cells may have occurred in the presence of Ro 20-1724, resulting in either enhanced suppressor or reduced helper function. An additional possibility is that Ro 20-1724 effects on T cells are expressed only during the latter phase of the 10-day culture period when T cell helper function for IgE synthesis (late) may be more prominent. Conceivably, PDE inhibition of T cells at a later time in the culture may show more dramatic effects.

Using adherence methods, Romanagni et al [11] and Hsieh [29] examined the effect of monocyte depletion on IgE synthesis. Hsieh found that removal of adherent cells reduced IgE synthesis, but the study did not control for nonspecific loss of B cells during adherence. Preliminary experiments to examine the role of monocyte suppressive influences showed that depletion of monocytes by adherence to tissue culture flasks resulted in reduction of IgE production. This was in large part due to nonspecific loss of surface immunoglobulin (SIg^+) cells in plastic tissue culture flasks which occurred to a greater degree than in the Petri dishes used in panning. Reduced IgE production by MNL depleted of monocytes by adherence was not due to a change in IgE production per B cell (unpublished observations). Romagnani et al [11] also observed this decrease of IgE in MNL depleted of adherent monocytes but showed evidence that the reduction was in large part accountable by loss of preformed IgE, consistent with our above interpretation.

With non-IgE in vitro immunoglobulin production assays, the role of agents that alter cyclic nucleotides in the modulation of immunoglobulin production is unclear [30]. However, some investigators have been able to demonstrate that isoproterenol induced inhibition of immunoglobulin-secreting cells and that this effect was mediated through suppressor T-cell induction [30]. Since MNL from normal individuals and patients with AD spontaneously produce only minimal amounts of IgG, IgA, and IgM in vitro [20], the effects of Ro 20-1724 on spontaneous production of these allotypes remain to be investigated.

Despite the very low levels of IgG production in these unstimulated cultures, it is conceivable that part of the changes in total IgE synthesis observed here may be due to changes in idiotype contained within IgG, and for which the anti-IgE carries anti-idiotypic activity. However, the use of 2 independently generated anti-IgE antibodies reduces the likelihood of detecting common idiotypes in the radioimmune assay.

In conclusion, inhibition of the elevated cAMP PDE in MNL from patients with AD resulted in a closely related reduction in IgE synthesis by spontaneously producing B cells in vitro. The effect was predominantly mediated directly on B cells, but some inhibition was mediated indirectly through T cells as well. PDE inhibitors may provide an exciting new investigational probe and therapeutic tool in AD and allow us to firmly link the cyclic nucleotide and immunologic abnormalities in the disease.

The authors express their thanks to Lynn-Marie Dixon and Barbara Winters for their assistance in preparation of this manuscript.

REFERENCES

1. Johnson HH, DeOreo GA, Lascheid WP, Mitchell FJ: Skin histamine levels in chronic atopic dermatitis. J Invest Dermatol 34:237–238, 1960
2. Juhlin L: Localization and content of histamine in normal diseased skin. Acta Derm Venereol (Stockh) 47:383–391, 1967
3. Safka MH, Chan SC, Cooper KD, Hanifin JM: Heterologous desensitization of leukocytes: a possible mechanism of beta adrenergic blockade in atopic dermatitis. J Allergy Clin Immunol 68:218–225, 1981
4. Chan SC, Grewe SR, Stevens SR, Hanifin JM: Functional desensitization due to stimulation of cyclic AMP-phosphodiesterase in human mononuclear leukocytes. J Cyclic Nucleotide Res 8:211–224, 1982
5. Grewe SR, Chan SC, Safko MJ, Hanifin JM: Elevated leukocyte cyclic AMP-phosphodiesterase in atopic disease: a possible mechanism for cyclic AMP-agonist hyporesponsiveness. J Allergy Clin Immunol 70:452–457, 1982
6. Juhlin L, Johansson SGO, Bennich J, Hogman C, Thyresson N: Immunoglobulin E in dermatoses. Arch Dermatol 100:12–16, 1969
7. Butler J, Chan SC, Stevens S, Hanifin JM: Increased leukocyte histamine release associated with elevated cAMP phosphodiesterase activity in atopic dermatitis. J Allergy Clin Immunol 71:490–497, 1983
8. Sampson HA, Buckley RA: Human IgE synthesis *in vitro*: a reassessment. J Immunol 127:829–834, 1981
9. Saxon A, Morrow C, Stevens RH: Subpopulations of circulating B cells and regulatory T cells involved in *in vitro* IgE production in atopic patients with elevated serum IgE. J Clin Invest 65:1457–1468, 1980
10. Tjio AH, Hull WM, Gleich GJ: Production of human immunoglobulin E antibody *in vitro*. J Immunol 122:2131–2133, 1979
11. Romagnani S, DelPrete GF, Maggi E, Troncone R, Guidizi GM, Almerigogna F, Ricci M: *In vitro* production of IgE by human peripheral blood mononuclear cells. II. Cells involved in the spontaneous IgE production in atopic patients. Clin Exp Immunol 42:579–588, 1980

12. Geha RS, Reinherz E, Leung D, McKee KT Jr, Schlossman S, Rosen FS: Deficiency of suppressor T cells in the hyperimmunoglobulin E syndrome. J Clin Invest 68:783–791, 1981
13. Iwamoto I, Nawata Y, Yanagisawa T, Yoshida S, Itaya T, Tomioka H, Kumagai A: *In vitro* production of anti-mite IgE antibody and its suppression by human peripheral blood lymphocytes. Int Arch Allergy Appl Immunol 66:67–74, 1981
14. Saryan J, Leung D, Geha R: Induction of human IgE synthesis by a factor derived from T cells of patients with hyper-IgE states. J Immunol 130:242–247, 1983
15. Mage M, McHugh L, Rothstein T: Mouse lymphocytes with and without surface immunoglobulin: preparative scale preparation in polystyrene tissue coated with specifically purified immunoglobulin. J Immunol Methods 15:47–56, 1977
16. Wysocki L, Sato V: "Panning" for lymphocytes: a method for cell selection. Proc Natl Acad Sci USA 75:2844–2848, 1978
17. Engleman E, Benike C, Glickman E, Evans R: Antibodies to membrane structures that distinguish suppressor-cytotoxic and helper T lymphocyte subpopulations block the mixed leukocyte reaction in man. J Exp Med 153:193–198, 1981
18. Reinherz E, Penta A, Hussey R, Schlossman S: A rapid method for separating functionally intact human T lymphocytes with monoclonal antibodies. Clin Immunol Immunopathol 21:257–266, 1981
19. Cooper KD, Kang K, Hanifin JM: Direct depletion and purification of monoclonal antibody defined cells from unfractionated human mononuclear leukocytes using antibody coated polystyrene Petri dishes. J Clin Lab Immunol 13:97–100, 1984
20. Cooper KD, Kazmierowski JA, Wuepper KD, Hanifin JM: Immunoregulation in atopic dermatitis: functional analysis of T and B cell interactions and the enumeration of Fc receptor bearing T cells. J Invest Dermatol 80:139–145, 1983
21. Thompson WJ, Terasaki WL, Epstein PM, Strada SJ: Assay of cyclic nucleotide phosphodiesterase and resolution of multiple molecular forms of the enzyme. Adv Cyclic Nucleotide Res 10:69–92, 1979
22. Giustina TA, Butler JM, Chan SC, Stevens SR, Hanifin JM: Phosphodiesterase inhibitor effects on leukocyte enzymes and on histamine release in atopic dermatitis. Clin Res 30:164A, 1982
23. Goldstein G, Sheid N, Boyse A, Brand A, Gilmore D: Thymopoietin and bursopoietin: induction signals regulating early lymphocyte differentiation. Cold Spring Harbor Symp Quant Biol 41:5–9, 1977
24. Pahwa S, Pahwa R, Goldstein G, Good RA: Immunoregulatory influence of thymic epithelium and thymopoietin on human B lymphocyte differentiation. Cell Immunol 56:40–45, 1980
25. Wolfe R, Goldstein AL, Ziff M: Suppression by thymosin of pokeweed mitogen-induced differentiation of human B cells. Clin Immunol Immunopathol 11:303–310, 1978
26. Hamerling V, Chin A, Abbot J, Goldstein G, Sandberg M, Hoffman M: The ontogeny of B lymphocytes. III. Opposite signals transmitted to B lymphocyte precursor cells by inducing agents and hormones. Eur J Immunol 6:868–873, 1976
27. Cooper K, Chan SC, Hanifin JM: Differential cAMP responsiveness in receptor binding by atopic T and B cells suggests altered immunoregulatory mechanisms. Clin Res 29:5, 1981
28. Etlinger HM, Chiller JM: Induction of tolerance in athymic mice with an antigen which is highly immunogenic in euthymic mice. Cell Immunol 33:297–311, 1977
29. Hsieh K-H: Increased adherent suppressor cell activity in allergic children. Ann Allergy 47:186–188, 1981
30. Katz P, Fauci AS: Activation of human B lymphocytes. VII. The regulatory effect of cyclic adenosine monophosphate on human B cell activation. J Allergy Clin Immunol 61:334–338, 1978