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# Differential Effects of Interleukin-2 and Interleukin-4 on Immunomodulatory Role of Platelet-Activating Factor in Human B Cells

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**Platelet-activating factor (PAF), a naturally occurring phospholipid cytokine, is a potent mediator of allergic and inflammatory reactions, as well as a modulator of immune responses. In the present study we showed that PAF is involved in early B-cell activation, as demonstrated by the increased cyclic AMP (cAMP) generation by PAF in a time- and dose-dependent manner in anti-μ antibody- plus B-cell growth factor-activated normal human peripheral blood B lymphocytes. PAF also regulated differentiation by causing a biphasic response on immunoglobulin M (IgM) production with an inhibitory signal generated at $10^{-6}$ M and a stimulatory signal generated at $10^{-8}$ to $10^{-10}$ M. PAF enhanced IgG and IgA secretion. The regulation exerted by PAF was shown to be specific because the addition of the PAF antagonist CV-3988 abrogated these effects and the inactive form of PAF, lyso-PAF, induced neither cAMP generation nor immunoglobulin secretion in normal human B cells. Other cytokines, interleukin-2 (IL-2) and IL-4, potent mediators of the immune response, were unable to elicit a cAMP response in B cells. However, the addition of PAF ($10^{-6}$ M) with either IL-2 or IL-4 enhanced cAMP production above the levels enhanced by the addition of PAF alone. IL-2 or IL-4, individually, stimulated IgM production, yet costimulation with PAF resulted in a differential effect between IL-2 and IL-4. PAF down-regulated the IL-4-induced IgM secretion, whereas the IL-2-induced IgM secretion was enhanced. The presence of CV-3988 returned all values to those obtained with IL-2 or IL-4 alone, demonstrating the specificity of PAF. These data suggest that PAF is an important B-cell immunomodulator which can interact with other leukocyte cell mediators.**

The differentiation of human B cells into immunoglobulin-secreting cells involves a cascade of cellular events that leads the cell through activation, proliferation, and finally, differentiation (2). This can be achieved by cross-linking of the antigen receptor with anti-μ antibody, which in turn causes the B cells to be responsive to two major lymphokines involved in the control of B-cell differentiation, interleukin-2 (IL-2) and IL-4 (8). Many lymphokines play a role in the maturation stages of the B cells, each of which can modulate the maturation process at a single stage or through several stages. These lymphokines interact with specific receptors on the B-cell membrane and modulate a cascade of intracellular events. This may then trigger the activation of enzymes to modulate second messenger molecules such as cyclic AMP (cAMP) and diacylglycerol which subsequently activate specific protein kinases such as protein kinase A (PKA) and protein kinase C (PKC), respectively, which, by phosphorylating their substrates, initiate other cascades of intracellular events (19).

Human IL-4 has been shown to have pleiotropic effects on the immune system; in particular, it serves as a growth factor for activated B cells and modifies immunoglobulin isotype production (11). Activation of human B cells by IL-4 results in their enlargement and induction of CD23 antigen, CD40 antigen, and soluble immunoglobulin M expression (10). IL-2 exerts direct effects on B cells such as enhanced proliferation and immunoglobulin secretion (20). B cells constitutively express mRNAs for IL-2 receptor α and β chains (IL-2Rα and IL2Rβ, respectively), thus establishing their potential to respond to IL-2 (29). Both IL-2 and IL-4 share many target cells and, in so doing, promote the growth and differentiation of lymphocytes.

IL-2 and IL-4 are not the only cytokines involved in the activation of B-cell events. Platelet-activating factor (PAF) is a potent ether phospholipid cytokine, a mediator of allergic and inflammatory reactions, as well as a modulator of immune responses. PAF has been shown to be generated by several types of inflammatory cells such as monocytes, macrophages, polymorphonuclear leukocytes, lymphocytes, platelets, and endothelial cells (40). PAF is responsible for the modulation of a wide range of immunological cellular events such as elevation of intracellular calcium levels (25, 52), liberation of arachidonic acid (44), phosphoinositide turnover (15, 45), up-regulation of the proto-oncogenes c-*fos*, c-*jun*, and *EGR2* (28, 44), as well as the induction of tyrosine phosphorylation of phospholipase C (24). PAF as well as IL-2 and IL-4 can modulate such cellular events as cAMP generation (9, 55), proliferation (3, 13), and immunoglobulin production (12). At this time there is no consensus of opinion on the action of PAF on these particular cellular events in B lymphocytes.

Because of the interactive nature of cytokines and the evidence in T cells of PAF regulation of IL-2- and IL-4-mediated cellular events (41), as well as studies showing IL-4-induced up-regulation of PAF receptor expression on B lymphocytes (30), we decided to investigate the modulatory effects of PAF on normal human B-cell activation and differentiation. We report that PAF modulates cAMP generation and immunoglobulin production in normal human B cells, additionally augmenting the IL-4- or IL-2-induced cAMP

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accumulation, but with a differential effect on IL-2- or IL-4-induced immunoglobulin M (IgM) production.

## MATERIALS AND METHODS

**Reagents.** The following reagents (and their sources) were used: low-molecular-weight B-cell growth factor (BCGF; Cellular Products, Buffalo, N.Y.), goat $F(ab')_2$ fragment to human IgM (5 Fc μ; Organon Teknika, Durham, N.C.), [$^3$H]cAMP Binding Protein Assay Kit (Diagnostic Products, Los Angeles, Calif.), 3-isobutyl-1-methylxanthine (IBMX; Sigma, St. Louis, Mo.), PAF (1-*O*-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine), CV-3988 (Biomol, Plymouth Meeting, Pa.), and recombinant IL-2 and IL-4 (Genzyme, Cambridge, Mass.).

**Culture medium.** The medium required for the isolation of B cells was RPMI 1640 supplemented with penicillin, streptomycin, glutamine, and heat-inactivated fetal bovine serum (FBS; GIBCO, Grand Island, N.Y.). All cultures were carried out in HB101 serum-free medium (Irvine Scientific, Santa Ana, Calif.) supplemented with penicillin, streptomycin, glutamine, sodium pyruvate, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; GIBCO), and 0.17% bovine serum albumin (BSA; Sigma). The total albumin content of the HB101 medium was 0.25%.

**B-cell purification.** B lymphocytes were positively selected from platelet-depleted buffy coats, obtained from the Gulf Coast Regional Blood Center, by the technique of Funderud et al. (17). The buffy coat was transferred to a 75-cm$^2$ cell culture flask and diluted with 25 ml of RPMI 1640 plus 0.01 M EDTA without FBS. Anti-CD19-coated magnetic beads (Dynabeads M-450; Dynal, Oslo, Norway) were added to the cell suspension in a target-to-bead ratio of 1:5. The mixture was incubated at 4°C for 30 min. The flask was then placed on a magnet for 5 min to allow for the collection of the CD19 bead–B-lymphocyte complex. The bead-cell complexes were collected and washed five times in RPMI 1640 containing 1% FBS by magnetization. The cells were then separated from the magnetic beads by the protocol of Rasmussen et al. (38). The bead-cell complexes were resuspended in a small volume of RPMI 1640 with 1% FBS, and a goat anti-mouse Fab antiserum (Detach-A-Bead; Dynal) was added at a ratio of 1 unit of Detach-A-Bead:100 μl of resuspended bead-cell complex. This mixture was rotated for 45 min at ambient temperature. The cells were then washed in RPMI 1640 with 1% FBS two times by magnetization, and the supernatants (containing the detached B lymphocytes) were collected. The population of B cells obtained following the final separation step from the Detach-A-Beads contained approximately 2 to 3% T cells and <1% monocytes as determined by staining with CD2 and CD14 (Becton Dickinson, Mountain View, Calif.); this was followed by analysis on the FACS (Epics Profile, Coulter, Hialeah, Fla.). The B-cell preparations contained >97% CD20 (Becton Dickinson)-positive B cells.

**Culture conditions.** All B cells were cultured for an initial incubation time of 48 h in the presence of a combination of BCGF (1:500) and anti-μ antibody (15 μg/ml) in RPMI 1640 plus 1% FBS at 37°C in a humidified atmosphere of 5% $CO_2$–95% air.

The choice of BCGF in combination with anti-μ antibody was based on the fact that anti-μ antibody alone stimulated resting B cells to enter only the $G_1$ phase. The addition of BCGF was necessary to drive the cells into the S phase and, thus, to sustain the long-term growth of anti-μ antibody-activated normal B lymphocytes (23).

At the end of this incubation time, cells were washed in HB101 with 0.17% BSA and were resuspended in fresh medium. Depending upon which assay system was used, the cells either were immediately prepared for assay in cAMP experiments or were recultured in the presence of the appropriate stimuli for immunoglobulin determination. Cultures for immunoglobulin studies were carried out in triplicate, with each tube containing $5 \times 10^5$ B cells per ml of HB101 with 0.17% BSA. The cells were then incubated for a final 7 days at 37°C. Since PAF was dissolved in ethanol prior to dilution in culture medium, control cultures contained ethanol at an equivalent maximal concentration, and the ethanol had no effect in the assays used in the present study.

**cAMP determination.** The incubation medium contained cells diluted to a final concentration of $4 \times 10^6$ cells per ml in HB101 plus 0.17% BSA with 25 mM HEPES buffer (pH 7.4). The cell cultures were preincubated for 15 min at 37°C in the presence of 1 mM IBMX, a phosphodiesterase inhibitor, after which time the test agents were added and the incubation was continued for the appropriate length of time. The reaction was terminated by centrifugation, and cAMP was extracted from the cells by the addition of 95% acidic ethanol prior to homogenization. After washing, the supernatants were evaporated and the cAMP content was determined by using a competitive protein binding assay (46).

**Measurement of immunoglobulin synthesis.** Following 7 days of incubation, the supernatants from the cell cultures were harvested and frozen at −20°C until use. The immunoglobulin levels in the supernatants were measured by enzyme-linked immunosorbent assay (ELISA) by using the following methodology. Ninety-six-well flat-bottom plates were coated with either goat anti-human IgM, μ-chain specific for measurement of IgM production or goat anti-human IgG, γ-chain specific for measurement of IgG production or goat anti-human IgA, or α-chain specific for measurement of IgA production diluted in sodium bicarbonate buffer (pH 9.4) to a final concentration of 2 μg per well per 100 μl. The solutions to be assayed were incubated in 50-μl volumes diluted with 200 μl of 1% BSA in phosphate-buffered saline (PBS) for 1 h at room temperature. Plates were washed with 0.05% Tween 20 in PBS and were incubated for 1 h with alkaline phosphatase-conjugated goat anti-human IgM (μ-chain-specific), anti-human IgG (γ-chain-specific), or anti-human IgA (α-chain-specific) antiserum. Plates were washed again and were finally incubated for 30 min with 250 μl of 5 mM *p*-nitrophenylphosphate substrate in 1.0 M diethanolamine buffer at pH 9.8. The reaction was stopped with 250 μl of 1.0 M NaOH, and the samples were brought to a final volume of 2.0 ml with PBS. The $A_{405}$ was read by using a Gilford spectrophotometer, and the immunoglobulin contents of the supernatants were determined from the standard curve generated by using dilutions of normal human serum pools with known IgG, IgM, and IgA contents by using a Dynatech Immunosoft program (42).

**Statistical analysis of the data.** All statistical comparisons were made with Student's *t* distribution for unpaired data. Variation among similar experiments was assessed by analysis of variance (ANOVA) testing and was found to be statistically not significant.

## RESULTS

**PAF-enhanced cAMP generation in normal human B lymphocytes.** Treatment of normal human B cells with PAF resulted in enhanced cAMP generation in both a time- and dose-dependent manner. Immediately following B-cell isolation, the B cells were preincubated for 48 h at 37°C in the presence of both BCGF (1:500) and anti-μ antibody (15 μg/ml), after which time they were washed with the appropri-

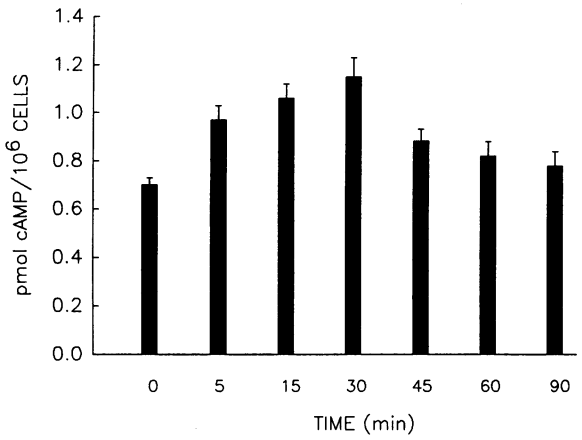


FIG. 1. Time course of cAMP accumulation in B cells incubated with PAF. BCGF plus anti-μ antibody-activated human B cells were incubated with $10^{-6}$ M PAF in the presence of 1 mM IBMX. The reaction was terminated at the indicated times, and the cells were assayed for their cAMP contents. Results are expressed as means ± standard errors of at least two experiments performed in triplicate.

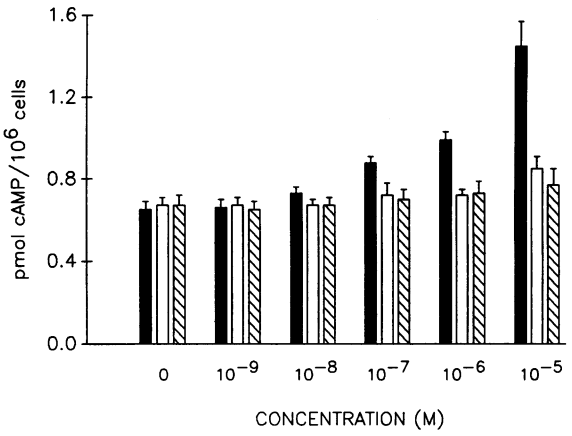


FIG. 2. Dose-response curve and specificity of PAF on normal human B-cell cAMP generation. B cells were preincubated with BCGF (1:500) and anti-μ antibody (15 μg/ml) for 48 h. IBMX was present at 1 mM throughout the incubation. PAF (■) or lyso-PAF (▨) (the inactive form of PAF) at various concentrations was incubated with 4 × $10^6$ B cells per ml of HB101 with BSA and HEPES for 30 min at 37°C. Lyso-PAF exerted no significant effect over the effect found at the baseline (0.7 pmol/$10^6$ cells). Inhibition of PAF-induced cAMP accumulation was assessed by simultaneous incubation of various concentrations of PAF with the PAF receptor antagonist CV-3988 ($10^{-6}$ M) (□). CV-3988 suppressed the PAF-induced increase in cAMP generation without any effects over the effect found at the baseline (no added PAF). The results for the PAF dose-response curve are expressed as means ± standard errors of six independent experiments performed in triplicate; all others are expressed as means ± standard errors of two independent experiments performed in triplicate. Variation among similar PAF doses was found to be not significant, as determined by ANOVA testing.

ate assay medium and were resuspended in their final assay volume. At a concentration of $10^{-6}$ M, PAF exerted a stimulatory effect on B-cell cAMP generation by as early as 5 min, with optimal levels of enhanced cAMP production occurring at 30 min; this was followed by a subsequent return to baseline levels by 60 min (Fig. 1).

Normal human B cells were incubated with PAF for 30 min at 37°C. This resulted in cAMP levels enhanced significantly above the baseline levels at concentrations of $10^{-5}$ M ($P < 0.001$), $10^{-6}$ M ($P < 0.001$), and $10^{-7}$ M ($P < 0.05$) (Fig. 2).

**Specificity of the effect of PAF on normal human B-cell cAMP generation.** The specificity of the effect of PAF on normal human B lymphocytes was verified by the addition of lyso-PAF, the inactive precursor, under conditions identical to those described above for PAF alone. Figure 2 shows that the presence of lyso-PAF exerted no significant effect on cAMP accumulation over the effect found at the baseline.

Additionally, the specificity of this effect of PAF was assessed by the addition of a PAF receptor antagonist, CV-3988, to the cultures. This particular PAF receptor antagonist was chosen from a variety of potential antagonists because of its close similarity to the chemical structure of PAF, which the other PAF antagonists lack (47). CV-3988 alone, at a concentration of $10^{-6}$ M, exerted no significant effect on cAMP baseline levels. CV-3988, in the presence of various concentrations of PAF, abrogated the PAF-induced enhancement of B-cell cAMP generation to baseline or near baseline values (Fig. 2).

**Effect of PAF on immunoglobulin production.** The incubation of PAF with normal human B lymphocytes at 37°C for 7 days resulted in a biphasic response on normal human B-cell IgM production (Fig. 3). PAF at $10^{-6}$ M inhibited IgM synthesis by 79% below that of the values at the baseline ($P < 0.01$). Conversely, PAF at $10^{-8}$, $10^{-9}$, and $10^{-10}$ M enhanced IgM secretion 52, 47, and 59%, respectively ($P < 0.01$), above the values at the baseline, returning to baseline levels at a PAF concentration of $10^{-11}$ M.

Figure 4 demonstrates the specificity of the effects exerted by PAF on IgM production. Substitution of the inactive precursor lyso-PAF for PAF showed neither inhibition nor enhancement of IgM production. Further experiments to verify the specificity of PAF on IgM secretion involved the addition of a PAF antagonist, CV-3988 ($10^{-6}$ M), in the presence of various concentrations of PAF. CV-3988 inhibited both the inhibitory and stimulatory effects of PAF on IgM production.

The advantage of using normal human B cells is the fact that there is a heterogeneous population of B cells which are at different developmental stages; this occurs naturally in vivo. This allows for the measurement of the level of secretion of different immunoglobulin isotypes. We therefore measured IgA and total IgG production in the presence of various PAF concentrations. PAF enhanced both IgA and total IgG production in a dose-dependent manner. Optimal increases in IgA production were observed with $10^{-8}$ M PAF, with a 54% increase over that of the control ($P < 0.01$), whereas in IgG production, $10^{-7}$ M PAF showed the greatest effect, with a 103% increase over that of the control value ($P < 0.01$) (Table 1).

**Effect of PAF in the presence of IL-4 on cAMP production.** IL-4 alone (10 to 500 U/ml) had no significant effect on B-cell cAMP generation, as shown in Fig. 5. Because a time curve of IL-4 also indicated no effect on cAMP generation (data not shown), the 30-min incubation time was chosen on the basis of the maximal effect of PAF. The addition of PAF ($10^{-6}$ M) to IL-4-treated B lymphocytes caused a significant enhancement in cAMP generation above that at the baseline ($P < 0.01$). The combination of PAF with 10, 50, and 100 U of IL-4 per ml caused further increases in the cAMP contents of 27% ($P < 0.05$), 38% ($P < 0.01$), and 53% ($P < 0.01$), respectively, over that observed with PAF alone (Fig. 5).

This enhancement of B-cell cAMP production by PAF and IL-4 was shown to be a specific effect resulting from the

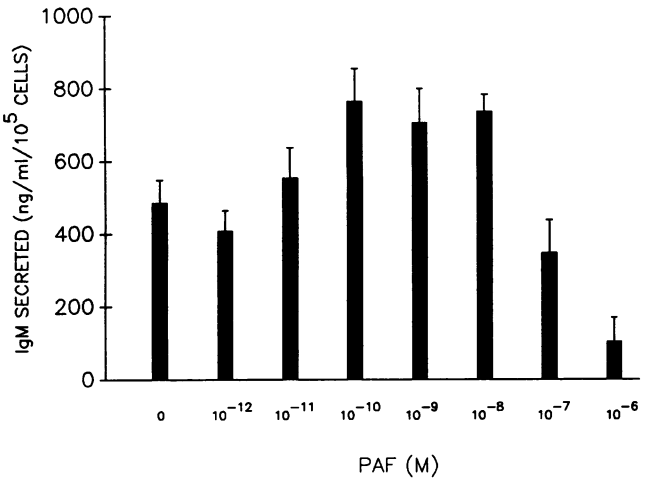


FIG. 3. Dose-response curve of IgM production of normal human B cells to PAF. A total of $5 \times 10^5$ B cells per ml of HB101 plus 0.17% BSA were preincubated with BCGF (1:500) and anti-μ antibody (15 μg/ml) for 48 h. The cells were washed and were subsequently recultured with or without various concentrations of PAF. After 7 days, the supernatants were harvested for IgM determination by ELISA. The results are expressed as the means ± standard errors of five independent experiments performed in triplicate. The variation among similar PAF doses was found to be not significant, as determined by ANOVA testing. PAF at $10^{-6}$ M significantly inhibited IgM production ($P < 0.001$), and PAF at between $10^{-8}$ to $10^{-10}$ M enhanced IgM production ($P < 0.01$).

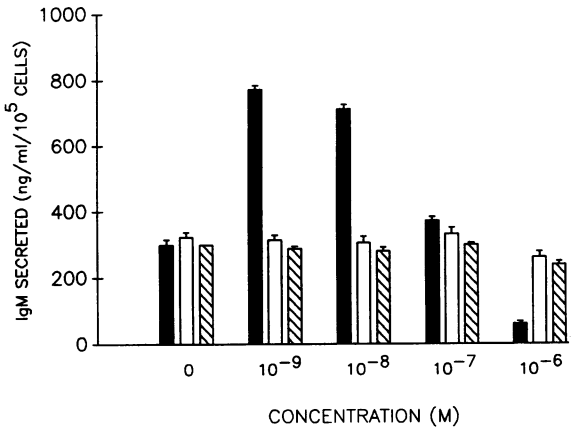


FIG. 4. Determination of the specificity of the effects of PAF on IgM production on normal human B cells. Assay conditions were similar to those indicated in the legend to Fig. 3. Lyso-PAF (▨), the inactive form of PAF, was added at concentrations of $10^{-9}$ to $10^{-6}$ M to $5 \times 10^5$ B cells per ml, as was PAF (■). Lyso-PAF exerted no significant effect on IgM production over the baseline level (300 ng/ml/$10^5$ cells). The results are expressed as means ± standard errors of five experiments performed in triplicate for PAF data and two experiments for lyso-PAF data. Suppression of PAF-mediated IgM secretion was determined by the simultaneous incubation of various PAF concentrations with the PAF antagonist CV-3988 ($10^{-6}$ M) (□). CV-3988 significantly inhibited the IgM responses mediated by PAF at $10^{-6}$, $10^{-8}$, and $10^{-9}$ M ($P < 0.01$). CV-3988 alone exerted no significant effect over baseline levels, as shown at the zero concentration point. The results are expressed as means ± standard errors of two experiments performed in triplicate.

presence of PAF. The addition of the PAF receptor antagonist CV-3988 suppressed the PAF-induced effects in the presence of IL-4 (10 to 500 U/ml), resulting in cAMP production equivalent to that at the baseline or with IL-4 alone (Fig. 5).

**Effect of PAF in the presence of IL-2 on cAMP generation.** As observed with IL-4, IL-2 (10 to 100 U/ml) also exerted no significant effect on B-cell cAMP production (Fig. 6). Over time, IL-2 also had no significant effect on cAMP production (data not shown). The addition of $10^{-6}$ M PAF along with various concentrations of IL-2 resulted in a phenomenon similar to that observed by the addition of PAF plus IL-4. The presence of both PAF and IL-2 increased cAMP generation in B cells over that at the baseline. PAF in combination with 10 or 50 U of IL-2 per ml caused significant increases in cAMP accumulation above that of PAF alone; 52% ($P < 0.001$) and 30% ($P < 0.01$), respectively (Fig. 6).

These PAF-related effects in the presence of IL-2 were proven to be specific events of PAF metabolism, as shown in Fig. 6. The addition of a PAF antagonist, CV-3988 ($10^{-6}$ M), to cells exposed to both PAF and IL-2 decreased cAMP generation to either the baseline levels or the levels obtained after exposure to IL-2 alone.

In summary, PAF alone stimulates B-cell cAMP production. IL-2 or IL-4, which themselves exerted no observable effect on cAMP production, in concert with PAF caused an augmentation of the cAMP content. These PAF-modulated events are specific, as evidenced by their inhibition with the PAF receptor antagonist CV-3988 and their nonresponse with an inactive PAF precursor, lyso-PAF. A similar phenomenon was observed in a previous study (33). Phorbol myristate acetate (PMA) alone exerted no effect on B-cell cAMP accumulation, yet the addition of a cAMP-enhancing agent such as cholera toxin or prostaglandin $E_1$ in the presence of PMA caused enhanced cAMP production above that caused by either agent alone.

**Differential effect of PAF on cytokine-induced IgM production.** Normal human B cells were incubated for 7 days at 37°C in the presence of various concentrations of IL-4. The supernatants were then harvested for IgM determination. IL-4 significantly increased IgM synthesis ($P < 0.01$). This enhancement was dose dependent from 10 to 1,000 U/ml. The greatest enhancement was observed at 1,000 U/ml, with an increase above control values of 103% ($P < 0.001$) (Fig. 7).

IL-4-induced IgE production in mononuclear cells has been shown to be inhibited by PAF. Since it was possible that PAF might exert an effect on the IL-4-induced IgM production, IL-4

TABLE 1. PAF enhances IgA and IgG production[a]

| PAF concn (M) | Immunoglobulin secreted (ng/ml/$10^5$ cells) | |
|---|---|---|
| | IgA | IgG |
| 0 | 115 ± 6 | 130 ± 5 |
| $10^{-12}$ | 132 ± 4 | 145 ± 6 |
| $10^{-11}$ | 130 ± 5 | 155 ± 7 |
| $10^{-10}$ | 137 ± 5 | 175 ± 10 |
| $10^{-9}$ | 145 ± 6 | 178 ± 11 |
| $10^{-8}$ | 177 ± 9 | 170 ± 11 |
| $10^{-7}$ | 127 ± 7 | 264 ± 17 |
| $10^{-6}$ | 111 ± 8 | 222 ± 15 |

[a] Purified human peripheral blood B cells ($5 \times 10^5$ cells per ml) were cultured in the presence of PAF. After 7 days, culture supernatants were harvested and assayed for their IgA and total IgG contents by ELISA. All cultures were done in triplicate. The data represent the means ± standard errors of five separate experiments. Variation among similar doses was determined to be not statistically significant as assessed by ANOVA testing.

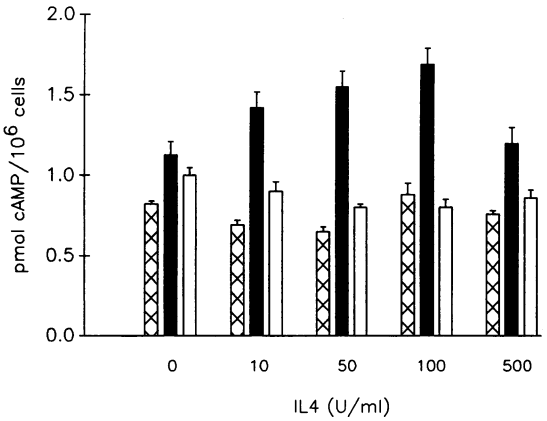


FIG. 5. Enhancement of PAF-induced B-cell cAMP generation by IL-4. Under conditions similar to those described in the legend to Fig. 1, B cells were incubated with various concentrations of IL-4 (⊠) for 30 min in the presence of 1 mM IBMX. IL-4 alone produced no significant effect on cAMP levels in B cells over those found at the baseline (0.82 pmol/$10^6$ cells). The costimulation of $10^{-6}$ M PAF with IL-4 (■) caused a significant increase over the stimulation found with PAF (1.15 pmol/$10^6$ cells) alone at IL-4 concentrations of 10, 50, and 100 U/ml ($P < 0.05$, 0.01, and 0.01, respectively). The values are represented as means ± standard errors of four independent experiments performed in triplicate. The specificity of this response was shown by the addition of $10^{-6}$ M CV-3988 to PAF plus IL-4 (□), which caused the cAMP levels to return to the baseline levels. Two experiments were performed in triplicate, and the values represent the means ± standard errors.

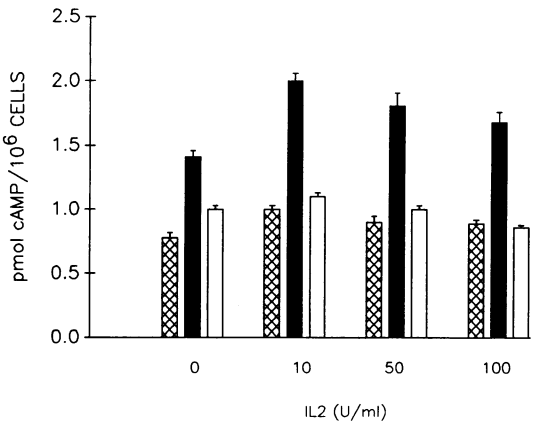


FIG. 6. Effect of IL-2 in the presence or absence of PAF and the subsequent inhibition by a PAF antagonist, CV-3988, on B-cell cAMP generation. Normal human B cells preincubated with BCGF (1:500) and anti-μ antibody (15 μg/ml) for 48 h were washed and incubated in the presence of 1 mM IBMX for 30 min at 37°C with either various concentrations of IL-2 alone (⊠) or IL-2 plus PAF ($10^6$ M) (■). IL-2 alone produced no significant change in cAMP levels over the levels found at the baseline (0.78 pmol of cAMP/$10^6$ cells). PAF ($10^{-6}$ M) increased cAMP levels to 1.4 pmol/$10^6$ cells. The addition of CV-3988 ($10^{-6}$ M) to cultures costimulated with IL-2 plus PAF (□) significantly inhibited the PAF plus IL-2-enhanced cAMP accumulation at all IL-2 concentrations assayed. Two experiments were performed in triplicate, and the values represent the means ± standard errors.

was added at various concentrations in the presence of PAF ($10^{-9}$ M). The concentration of $10^{-9}$ M PAF was chosen because it is physiologically relevant and it significantly enhanced IgM production. PAF ($10^{-9}$ M) significantly inhibited the IL-4-induced increase in IgM production at IL-4 concentrations of 100, 500, and 1,000 U/ml by 29% ($P < 0.05$) and 42 and 40% ($P < 0.01$), respectively, over the values of IL-4 alone (Fig. 7).

The addition of the PAF antagonist CV-3988 ($10^{-6}$ M) resulted in the return of IgM secretion to the levels obtained with IL-4 alone, thereby indicating that the PAF-mediated effects on IL-4-induced IgM production are specific (Fig. 8).

**PAF enhancement of IL-2-induced IgM secretion in normal human B cells.** B cells were cultured with or without PAF ($10^{-9}$ M) in the presence or absence of defined concentrations of IL-2. After 7 days, supernatants were harvested for IgM production. IL-2 alone caused significant increases in IgM production over that at the baseline, with the greatest increase of 134% observed at 100 U/ml ($P < 0.001$). The presence of PAF further enhanced the IL-2-induced IgM production at IL-2 concentrations of 10 and 50 U/ml by 49 and 67%, respectively ($P < 0.01$), over that of IL-2 alone (Fig. 9).

This effect by PAF on IL-2-induced IgM production was shown to be specific by the fact that CV-3988 ($10^{-6}$ M) caused a decrease in IgM production in cultures treated with IL-2 plus PAF to the levels obtained with IL-2 alone, thereby indicating that the enhancement was due to PAF (Fig. 10).

## DISCUSSION

The use of highly purified normal human B cells in our assay systems allowed for a more definitive assessment of the in vivo effects of modulators on specific B-cell events. Investigations on the role of PAF on events in lymphocytes have generally been carried out on lymphocyte populations that were either unfractionated (therefore predominantly T cells), altered by Epstein-Barr virus transformation, or tested in murine systems.

In the present study we showed that PAF stimulates cAMP generation in normal human B lymphocytes in a time- and dose-dependent manner as well as exhibits a dual response on

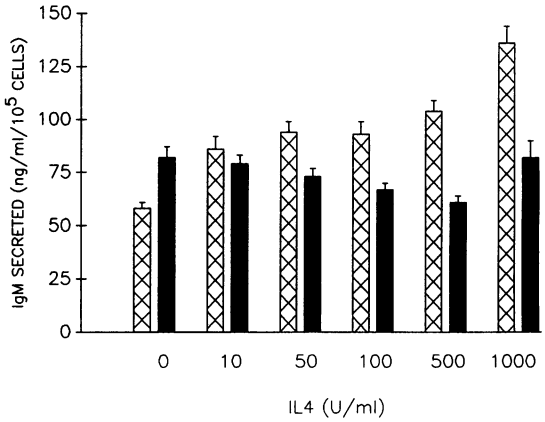


FIG. 7. Inhibition of IL-4-induced IgM secretion by PAF. Normal human B cells were preincubated with BCGF (1:500) plus anti-μ antibody (15 μg/ml) for 48 h. The cells were collected, washed, and subsequently recultured (day 0) with or without various concentrations of IL-4 (⊠) in the presence or absence of PAF ($10^{-9}$ M) (■). After 7 days, the supernatants were harvested for IgM determination by an ELISA. The data are represented as means ± standard errors of five separate experiments performed in triplicate. Variation among similar doses was found to be not statistically significant by ANOVA testing. IL-4 alone significantly increased IgM production at all concentrations assayed ($P < 0.01$). The addition of PAF ($10^{-9}$ M) (82 ng/ml/$10^5$ cells) significantly inhibited the IL-4-induced IgM response at 50 to 1,000 U of IL-4 per ml ($P < 0.01$).

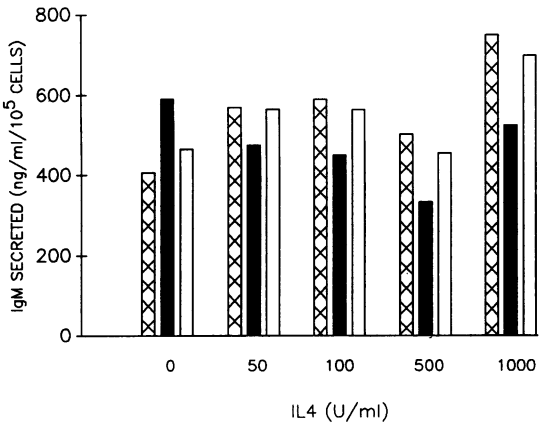


FIG. 8. Abrogation of IgM inhibition by PAF and IL-4. Assay conditions were identical to those indicated in the legend to Fig. 7. PAF ($10^{-9}$ M) in the presence of IL-4 (■) at the indicated concentrations caused an inhibition in IgM secretion. IL-4 alone (⊠) caused enhanced IgM production. This inhibition was reversed by the presence of CV-3988 ($10^{-6}$ M) (□). The data presented here are from a single experiment obtained by using triplicate samples and are representative of the results of three separate experiments.

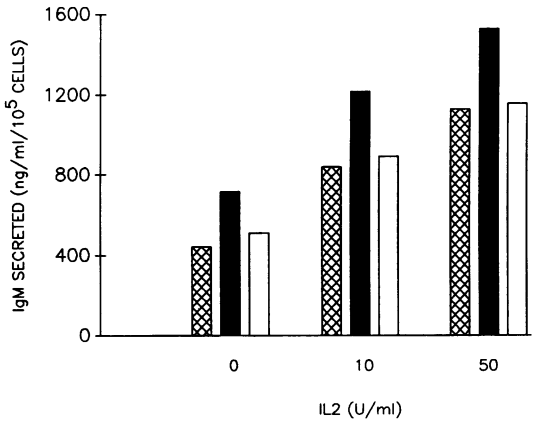


FIG. 10. Suppression of PAF plus IL-2-enhanced IgM secretion. Assay conditions were identical to those indicated in the legend to Fig. 9. PAF ($10^{-9}$ M) in the presence of IL-2 (■) at the indicated concentrations produced a further increase in IgM production over that induced by PAF or IL-2 alone (⊠). This enhancement was inhibited by the incubation of CV-3988 ($10^{-6}$ M) with PAF and IL-2 (□). The data presented here are from a single experiment obtained by using triplicate samples and are representative of the results of four separate experiments.

IgM production and enhanced IgG and IgA secretion. These PAF-induced events were shown to be specific by the absence of such effects in the presence of the PAF antagonist CV-3988 or the inactive form, lyso-PAF. Even though neither IL-4 nor IL-2 was capable of generating a cAMP response alone, the addition of PAF with either IL-4 or IL-2 resulted in enhanced cAMP accumulation, whereas PAF effected differential modulation on IL-4- or IL-2-induced IgM production.

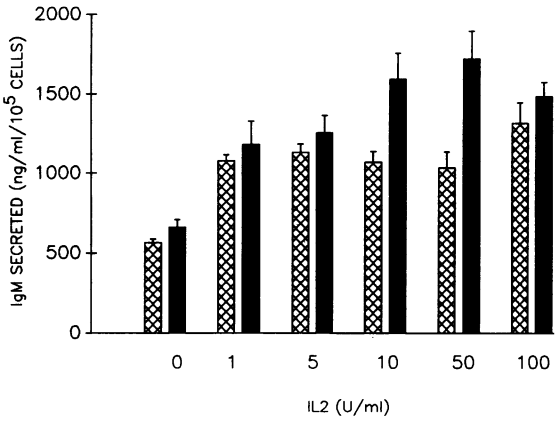


FIG. 9. Modulation of the IL-2-mediated enhancement of IgM secretion by PAF. Normal human B cells were preincubated with BCGF (1:500) plus anti-μ antibody (15 μg/ml) for 48 h. The cells were collected, washed, and subsequently recultured (day 0) with or without various concentrations of IL-2 (⊠) in the presence or absence of PAF ($10^{-9}$ M) (■). After 7 days, the supernatants were harvested for IgM determination by an ELISA. The data are represented as means ± standard errors of four separate experiments performed in triplicate. Variation among similar doses was found to be not statistically significant by ANOVA testing. IL-2 alone significantly increased IgM production at all concentrations assayed ($P < 0.001$). The addition of PAF ($10^{-9}$ M) significantly enhanced the IL-2-induced IgM response at 50 to 100 U of IL-2 per ml over that of PAF alone (661 ng/ml/$10^5$ cells) ($P < 0.01$).

Because PAF has been suggested to exert biologic properties that may be relevant to the pathogenesis of asthma and because cAMP analogs are bronchodilators which antagonize PAF during allergic bronchoconstriction, it is possible that there is a PAF-mediated cAMP involvement in the regulation of this immunologic disease. PAF has been shown by others to both decrease and increase cAMP levels in human unfractionated peripheral blood lymphocytes (36) and mononuclear cell preparations (43, 56) as well as to have a biphasic response on human peritoneal macrophages (1). It has been observed in neutrophils that cAMP inhibits PAF synthesis (16), and in monocytes cAMP causes the down-regulation of human PAF receptor mRNA at transcriptional and posttranscriptional levels (50). Thus, if these events are elucidated in B cells as well, in concert with the present findings, it may be that there is a PAF response to inflammation based on cAMP regulation.

PAF is released after antigenic challenge and may be involved in immediate hypersensitivity reactions, anaphylactic shock, or bronchoconstriction (4). PAF may also have a role in other immune responses, as observed in our system. PAF exerted dual biologic activities on IgM production on normal human B cells. A high concentration of PAF ($10^{-6}$ M) caused an inhibition in IgM secretion, whereas the more physiologically relevant PAF concentrations of $10^{-8}$ to $10^{-10}$ M produced enhanced IgM secretion, with a return to control values at $10^{-11}$ M. This biphasic response to PAF was not observed with the other isotypes assayed. This may be attributable to the fact that ours is a heterogeneous B-cell population in which subpopulations of B cells coexist. Each B-cell subset may respond differently to specific mitogenic stimuli and produce various cytokine-induced responses (10, 20). IgA and total IgG production was augmented by PAF.

These findings are in direct contrast to those of Deryckx et al. (12), who observed no effect of PAF on IgM, IgG, or IgA production. Those investigators used mononuclear cells (with a very high T-cell content), which were cultured for the entire incubation period in the presence of IL-4, thus possibly accounting for the discrepancy between their results and ours. Mazer et al. (27), who used lymphoblastoid B cells, showed

that PAF at concentrations similar to those that we used potentiated IgG production. The present study, however, is the first to demonstrate the effect of PAF on immunoglobulin production in normal human B cells.

The effects of PAF on immunoglobulin production were shown to be specific, as evidenced by (i) the lack of cytotoxic effects, measured by trypan blue exclusion, on the cells at the time of harvest (data not shown); (ii) the inability of the inactive form of PAF, lyso-PAF, over a wide concentration range to modulate immunoglobulin secretion; (iii) the fact that CV-3988, a structural analog antagonist of PAF, was an effective antagonist of PAF; and (iv) the fact that C-PAF, a potent, nonmetabolizable synthetic analog of PAF (31), yielded results similar to those obtained with PAF (data not shown).

Previous reports from our laboratory presented evidence of an inverse relationship between cAMP generation and immunoglobulin secretion (32, 46). Similarly, we showed that PAF is capable of exerting an inverse relationship between cAMP generation and immunoglobulin secretion by normal human B cells, as seen when $10^{-6}$ M PAF caused a significant enhancement in cAMP production with a corresponding inhibition of IgM production.

IL-4 is a well-documented stimulus for IgE production (34), yet there appears to be some controversy regarding the role of IL-4 in the production of other immunoglobulin isotypes. IL-4 has been shown to stimulate, inhibit, and even exert no response on the secretion of various immunoglobulin isotypes (IgM, IgG, IgA) (35, 39). The presence of either cytokine, IL-4 or IL-2, in our B-cell cultures stimulated IgM production. The IL-2-induced immunoglobulin production reported here is consistent with that found in other B-cell systems (7, 48). Incubation of BCGF plus anti-$\mu$ antibody-activated normal human peripheral blood B cells with IL-4 or IL-2 for up to 90 min resulted in no effect on cAMP generation. Other investigators reported somewhat varied effects of IL-4 on B cells (14, 18, 49). The variations noted among B-cell studies involving PAF, IL-2, or IL-4 may be due to the choice of separation technique, the use of the activated versus the quiescent state of the B cells, or the use of different mitogenic stimuli.

The data reported here not only demonstrate the response of B cells to these cytokines but the interactive modulation between them as well. The costimulation of cells with PAF and either IL-2 or IL-4 produced a substantial increase in cAMP generation. PAF and IL-2 costimulation also resulted in an augmentation of IL-2-induced IgM secretion, whereas there was an inhibitory effect exerted by PAF on the IL-4-induced IgM production in these cells.

Evidence exists for the differential effects of IL-2 and IL-4 on various cellular events such as proliferation (54), immunoglobulin production (21), calcium flux (22), cAMP generation (22), and IL-6 production (53). The differential effects observed in our system are thus not unique. There are reports of cellular events whereby IL-2 or IL-4 respond similarly but are mediated differentially by a common agonist. IL-2- or IL-4-induced B-cell proliferation has been shown to be differentially regulated in the presence of PKC inhibitors and calcium ionophores (53) and anti-CD23 (26).

The mechanism by which PAF achieves its effects on normal human B cells is unclear at this point. Given that PAF has been shown by us and others to modulate B-cell events, it may act directly on the B cell without a required involvement of granulocytes or T cells and their factors. Signaling by PAF could occur via a receptor-linked G protein, as evidenced by the fact that the PAF receptor is coupled to phospholipase C via a G protein (5). It also appears that there are different types of PAF receptors: those that are PKA responsive and those that are PKC responsive (6). This, in conjunction with the evidence that IL-4 responses are PKC independent and IL-2 responses are PKC dependent (23), may offer a possible explanation for the differential responses of PAF on the effects of IL-2 and IL-4 on normal human B cells.

In order to discern the mechanism of action operating in the system described here, further work is required in the area of protein kinase involvement and the potential for reversal of the differential effects. Other lymphokines such as gamma interferon and IL-1, which have been shown to reverse IL-2- and/or IL-4-regulated inhibition, may play a similar role in our system (37, 51).

In summary, PAF enhances both cAMP production and immunoglobulin secretion in normal human B cells and modulates IL-2 and IL-4-mediated B-cell cAMP and IgM responses. The role of PAF as a potent immunoregulatory agent appears to be of an interactive nature with other cytokines, thus mediating inflammatory, allergic, and immune reactions.

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