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Selective Inhibition of Polymorphonuclear Leukocytes by Immunosuppressive Concentration of Prostaglandin E_2

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Prostaglandin E_2(PGE_2) has been implicated as an immunosuppressive agent and plasma levels of PGE_2 are elevated in patients sustaining thermal injury. We examined the effect of 10^{-7}M prostaglandin E_2(PGE_2) on human polymorphonuclear leukocytes(PMN) to determine whether it directly inhibits stimulated responses of these cells. At this concentration, PGE_2 alone was incapable of stimulating PMN intracellular hydrogen peroxide production(indirectly assayed by fluorescence of 2',7'-dichlorofluorescin) or expression of the PMN CD11b/CD16 surface glycoproteins. PMN incubated in the presence of the soluble stimuli phorbol myristate acetate(PMA, 100 ng/ml) or recombinant human C5a(rHC5a, 10^{-8} M) generated significant amounts of hydrogen peroxide, increased their CD11b expression and decreased their CD16 expression. Pre-incubation of cells with PGE_2 caused significant inhibition of all the observed changes stimulated by rHC5a. In contrast, events stimulated by PMA were not affected by preincubation of cells with PGE_2. We conclude that PGE_2, in concentrations identical to those found in the plasma of patients with burn injuries, is capable of selectively inhibiting some stimulated events and phenotypic expression of PMN in vitro study.

Key Words: *PGE_2, Immunosuppression, Polymorphonuclear leukocytes, Hydrogen peroxide, CD11b, CD16*

INTRODUCTION

Human polymorphonuclear leukocytes(PMN) contain on their surface a glycoprotein, identified from within the integrin supergene family as the receptor for C3bi(CR3)(Fearson, 1980). CR3 increases adherence of PMN to endothelial surfaces and has been classified as an adhesion glycoprotein(Hynes 1987). This protein is a heterodimer composed of a 170kD α-chain(CD11b) and an invariant 95kD β-chain(CD18). Also contained on the PMN surface are several receptors for the Fc portion of immunoglobulins(FcgR). In particular, the 50-70 kD FcgR III is a low affinity receptor that binds immune-complexed IgG and has been designated CD16(Fleit et al., 1984 and Wright et al., 1986).

Together, these two surface proteins have been used to identify phenotypic subclasses of PMN. It has been reported that septic post burn-injured patients display lower levels of the CD11 + and CD 16 + in their circulation(Babcock et al., 1990). Recent in vitro

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studies have indicated that the CD16 antigen is shed into the supernatant from PMN subjected to soluble chemotactic stimuli(Huizinga et al., 1989a). The studies have indicated that expression of CD11b/CD18 measured by flow cytometric technique is increased following similar stimulation(Kishimoto et al., 1989). It is not understood what factors or events lead to these changes in CD11b/CD16 expression clinically.

Intracellular levels of hydrogen peroxide(H_2O_2) can reflect the level of oxidative burst activity of PMN that have been stimulated with soluble stimuli(Bass et al., 1986). It has also been reported that PMN taken from post-traumatic patients are less capable of generating oxidants that might participate in the killing of invading microorganisms(Faist et al., 1988). The nature of this immunosuppression is also not understood.

Following injury, prostaglandin E_2(PGE_2) is released from stimulated mononuclear cells. Distinct levels(1.0-3.0 ng/ml) have been assayed from the serum of burn patients(Ninnemann and Stockland, 1984) and from post-traumatic patients wherein PGE_2 suppresses interleukin-2 production by T lymphocytes(Faist et al.,1987). In mice, macrophages decrease their expression of the Ia histocompatibility antigen in the presence of similar concentrations of PGE_2(Snyder et al., 1982). Less is known regarding the effect of PGE_2 on the function of PMN. In this study, we report on the effects of immunosuppressive levels(10^{-7} M) of PGE_2 upon normal human PMN utilizing measurements of basal and stimulated CD11b and CD16 antigen expression and an assay for stimulated levels of intracellular H_2O_2.

MATERIALS AND METHODS

Monoclonal antibodies

Monoclonal antibody(mAb) Leu-15(IgG2a k) binds to the 170kD CD11b subunit and is a phycoerythrin(PE) conjugate(Ross et al., 1985). mAb Leu 11a(IgG1 k) binds to the 50-70kD CD 16 FcgR III and is a fluorescin isothiocyantae(FITC) conjugate. mAb 3G8(IgG1) also binds to CD16(Freeman and Shelby, 1988). For isotype controls, we used a mixture of mAb directed against the irrelevant keyhole limpet hemocyanin(KLH) labeled with FITC(IgG1 k) and PE(IgG2a k). For indirect staining(mAb 3G8) we used goat anti-mouse IgG that is a FITC conjugate. All antibodies were obtained from Becton-Dickinson(San Jose, CA, U.S.A.) except for mAb 3G8 which was a kind gift of Dr. Jan K. Horn, Department of Surgery at San Francisco General Hospital, San Francisco, CA, U.S.A.

Buffers and cell preparation

For each experiment, 10mM phosphate buffered normal saline(PBS), pH 7.4, containing 1.0 %(wt/vol.) gelatin and 5.0mM glucose(PBSg) was freshly prepared. For experiments involving PMN stimuli, the PBSg contained 1.0 mM $CaCl_2$ and 0.6 mM $MgCl_2$(PBSg++). For preparing WBC mixtures, ten ml venous blood was drawn using aseptic technique from normal volunteers into tubes containing ethylenediaminetetraacetic acid(EDTA) as anticoagulant. Lysis of red blood cells(RBC) was performed by mixing 0.80 %(wt/vol.) ammonium chloride containing 1.0 %(wt/vol.) $KHCO_3$ and 0.1 mM EDTA with anticoagulated blood(14 : 1) at room temperature(RT) for 3-5 min. The cells were then centrifuged at 300 × g for 5 min. at RT and the supernatant was discarded. The cell pellet was resuspended in cold PBS and washed at 4℃. Finally, the cells were resuspended in PBSg++ for stimulation.

For some experiments(Boyum, 1968), purified PMN were isolated by a modification of the method of Boyum. Briefly, RBC were removed by sedimentation with 6 %(wt/vol.) dextran in normal saline and hypotonic lysis with distilled H_2O. Washed cells were carefully under layered with 1.0 ml Ficoll-Hypaque(Pharmacia, Piscataway, NJ, U.S.A.) and centrifuged at 400 × g for 20 min. at RT. The bottom PMN pellet, obtained from the Ficoll-Hypaque step, was washed once with cold PBS. The cells were then resuspended in PBSg ++ for stimulation.

To determine viability, a 25 μl aliquot of the cell suspension was mixed with 25 μl of 0.0001 %(wt/vol.) ethidium bromide/0.0003 %(wt/vol.) acridine orange in PBS. Using fluorescent microscopy, live(green fluorescent) and dead(orange fluorescent) cells were counted and viability expressed as percent of total.

To avoid cell clumping following stimulation, EDTA was employed in the final suspensions for flow cytometric analysis. Cell centrifugation and washing was limited only to the period following termination of reactions in order to minimize handling artifacts.

Preparation of soluble stimuli

Phorbol myristate(PMA, Sigma, St Louis, MO) was

solublized in 95 %(vol./vol.) ethanol at a concentration of 20 mg per 10 ml and diluted with working buffer(PBSg++) to a stock of 1.0 μg/ml. The final concentration of ethanol exposed to cells was 0.01 %(vol./vol.). Recombinant human C5a(rHC5a, Sigma) was reconstituted and diluted to a stock concentration of 10^{-7} M with working buffer. PGE_2(Sigma) was diluted in working buffer to a stock concentration of 1.4×10^{-6} M. All stimuli diluted to their stock concentration were aliquoted into 0.5 ml volumes and stored for future use at -20℃. In each case, addition of stimuli to cell suspensions produced a 1 : 10 dilution of their final concentration.

CD11b / CD16 expression

Measurement of expression was performed on cells isolated from lysed whole blood. Cells(10^6 PMN/ml PBSg++) were exposed to buffer alone(control) or to soluble stimuli(final concentration) : PGE_2(10^{-7} M), PMA(100 ng/ml), or rHC5a(10^{-8} M) either alone or in sequence and incubated at 37℃ with agitation for the indicated time periods. Controls were included for the ethanol solvent of the PMA. Reactions were terminated by addition of an equal volume of cold(4℃) PBSg containing 1 % paraformaldehyde(Eastman Kodak, Rochester, NY, U.S.A.). The cells were then pelleted by centrifugation at 300 × g for 15 min at 4℃ and washed with PBSg. Aggregates were removed from FITC conjugates by centrifuging at 100,000 × g for 10 min prior to use. Cell pellets(10^6 PMN) were resuspended in 100 μl total volume of PBSg containing 10 μl of the appropriate mAb. Following incubation for 30 min at 4° C, 1.0 ml of cold PBSg was added and the mixture was centrifuged at 300 × g for 5 min at 4℃. For direct staining, the supernatant was aspirated and the cells were resuspended in 1.0 ml PBSg containing 0.5 % paraformaldehyde. For indirect staining, an additional incubation of cells in 100 μl PBSg containing 10 μl of FITC-conjugated goat anti-mouse IgG was performed for 30 min. at 4℃ prior to washing and final suspension in 0.5 % paraformaldehyde. For controls in direct staining, monoclonal antibodies of the same immunoglobulin isotype directed against irrelevant antigens(KLH) were used to characterize non-specific binding. In the case of indirect staining, a control of FITC-conjugated goat anti-mouse IgG binding to cells not exposed to the mouse mAb was used.

Intracellular hydrogen peroxide production

Measurement of intracellular H_2O_2 production was performed according to the method of Bass(Bass and Parce, 1983).

This assay relies upon the fact that the florochrome(2′,7′-dichlorofluorescin) is trapped within the cell and unable to leak out. Total cell fluorescences indirectly reflects intracellular hydrogen peroxide production and were measured by flow cytometry.

Cells isolated as described above were suspended(10^6 PMN/ml) in PBSg++ and preincubated with 5.0 μM 2′,7′-dichlorofluorescin diacetate(DCFH-DA, Eastman Kodak) in a final volume of 1.0 ml with horizontal agitation at 37℃ for 15 min. Cells were then exposed to buffer alone(control) or to soluble stimuli(final concentration) : PGE_2(10^{-7} M), PMA(100 ng/ml), or rHC5a(10^{-8} M) either alone or in sequence and incubated at 37℃ with agitation for the indicated time periods. Reactions were terminated by addition of 1.0 mM EDTA. Where appropriate, controls were included for the ethanol solvent of the PMA.

Flow cytometric analysis

Cells were analyzed by quantitative flow cytometry using a Becton-Dickinson(San Jose, CA, U.S.A.) FACScan, equipped with an argon laser emitting light at 488 nm. Forward angle light scatter and right angle light side scatter measurements were used to differentiate PMN from debris, cellular clumps, RBC, and other WBC. Electronic gates were established to include>95 % PMN. We collected logarithmic list mode data for each sample on 10,000 events within the PMN gate. The fluorescence of the cells was measured by photo multiplier tubes after light passed through a 525 NM band pass filter for FITC-stained cells, and a 575 NM band pass filter for PE-stained cells. Single parameter histograms were analyzed using Consort 30 software running on a Hewlett-Packard series 9000 computer.

Data analysis and statistics

All datas are expressed as mean ± SEM(mean standard error). Mean differences between experimental groups were compared by Student's t-test and differences with p values less than 0.05 were accepted as significant.

RESULTS

Following isolation, yield of WBC ranged from 4.0 to 8.8 × 10^6 cells/ml whole blood. Viability for the cell mixtures ranged from 93 to 98 %. PMN accounted for 46 to 68 % of the WBC mixtures. For most experiments we employed WBC mixtures to minimize prestimulatory cell handling. In Table 1 are displayed results of one experiment that compares the oxidative response of a WBC mixture isolated by ammonium chloride lysis with purified PMN obtained by standard Ficoll-hypaque separation. The observed levels of oxidative activity for the two populations appeared similar.

The time course for CD11b expression(stained with the PE-conjugated mAb Leu-15) by PMN is displayed in Fig. 1 and for CD16 expression(stained with the FITC-conjugated Leu-11a) is displayed in Fig. 2 as the mean cell fluorescence exhibited by the mode value for each population of PE-stained cells. For these experiments cells were incubated with stimuli for the indicated time periods and reactions were terminated by addition of an equal volume of cold 1.0 % paraformaldehyde. Cells were then stained directly with the PE-conjugated mAb Leu-15 or FITC-conjugated Leu-11a as described above. Basal CD11b/CD16 expression for PMN was determined by immediate fixation of isolated cells following lysis at 4℃. Extremely low levels of nonspecific binding were observed with all populations of cells tested for both immunoglobulin isotypes of KLH(mean fluorescence < 4.8 for IgG2a/PE-conjugated and mean fluorescence

Table 1. Effect of Cell Isolation Technique on Neutrophil Oxidation.

Preparation	Treatment[1]	Fluorescence[2]
Ammonium chloride lysis	none	1.1
	37℃	87.4
	PMA(100 ng/ml), 37℃	1882.5
Ficoll-Hypaque	none	1.4
	37℃	110.1
	PMA(100 ng/ml), 37℃	1723.5

[1]Following isolation, cells were incubated with 2',7'-dichlorofluorescin diacetate for 15 min at 37℃ and then further incubated with the indicated treatment for 15 min. Reactions were terminated by NaN_3.

[2]Mode values for fluorescence of PMN intracellular 2',7'-dichlorofluorescin obtained from one single experiment.

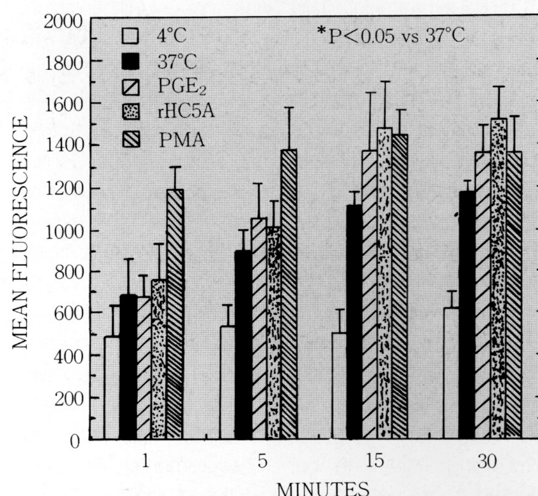

Fig. 1. Time course of CD11b expression by PMN. Data from three separate experiments is expressed as mean(± SEM) fluorescence of mode values for PMN. Following incubation for the times indicated, cells exposed to various conditions(see legend) were fixed and subjected to indirect immunofluorescent staining with phycoerythrin-conjugated anti-CD11b monoclonal antibody.

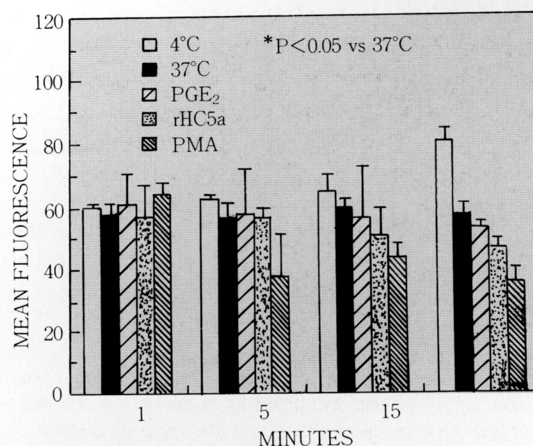

Fig. 2. Time course of CD16 expression by PMN. Data from three separate experiments is expressed as mean(±SEM) fluorescence of mode values for PMN. Following incubation for the times indicated, cells exposed to various conditions(see legend) were fixed and subjected to indirect immunofluorescent staining with phycoerythrin-conjugated anti-CD16 monoclonal antibody.

<5.4 for IgG1/FITC-conjugate).

Incubation at 37℃ produced a steady increase in CD11b expression. This was not significantly increased by addition of PGE_2 to the mixture, but was augmented with both C5a and PMA by 15 min. In contrast, CD16 expression was relatively stable with incubation at 37℃, and loss of expression occurred with stimulation by either rHC5a or PMA. Control incubations with the diluent of the soluble stimuli(ethanol) at identical concentrations as those in the experiments involving stimuli did not show any significant change in H_2O_2(data not shown).

In the course of our measurements of CD16 expression, we noted that the total mean fluorescence displayed by all populations of PMN tested was lower than that observed for CD11b, for example. To rule out the possibility that a high signal/noise ratio was producing artifact in our measurements, we conducted a comparison of two different mAb recognizing CD16(Leu-11a and 3G8). The results in Table 2 are from one experiment that examined the expression of CD16 following stimulation of PMN stained with two different antibodies. It can be seen that the results expressed as percentage of the unstimulated cells are similar, whereas, mode fluorescence values recorded after staining with 3G8 were higher than those obtained after staining with Leu-11a. Nonspecific binding of goat anti-mouse FITC-conjugate was negligible(mean fluorescence<6.2).

We examined the effects of pre-incubation of cells in the presence or absence of 10^{-7} M PGE_2 prior to stimulation upon CD11b/CD16 expression. The results in Fig. 3 represent the mean net cell fluorescence for each population of PE-stained cells, while Fig. 4 displays the mean net cell fluorescence for each population of FITC-stained cells. PGE_2 markedly suppressed the increased expression of rHC5a-stimulated CD11b as compared to controls incubated at 37℃. In contrast the PMA-stimulated CD11b expression was unaffected by PGE_2. For CD16 expression, PGE_2 suppressed the loss of expression stimulated by rHC5a, while it failed to affect PMA-induced changes.

The time course for intracellular hydrogen peroxide production by PMN is displayed in Fig. 5 as the mean cell fluorescence for 2′,7′-dicholorofluorescin.

Table 2. Results of Staining with Different anti-CD16 Monoclonal Antobodies.

Monoclonal Antobody	PMN Treatment[1]	Fluorescence[2] (Mode)	Percent Control
Leu-11a	4℃	100.9	-
	37℃	85.0	84.2
	$PGE_2(10^{-7}$ M)	88.1	87.3
	PMA (100 ng/ml)	12.8	12.7
3G8	4℃(control)	556	-
	37℃	481.2	86.5
	$PGE_2(10^{-7}$ M)	517.3	93.0
	PMA (100 ng/ml)	140.9	25.3

[1]Following isolation from ammonium chloride-treated whole blood, 10^6 PMN were incubated for 15 min in the absence-(control) or presence of stimuli, and then fixed by addition of an equal volume of 1.0% paraformaldehyde. Cells were then pelleted and subjected to direct or indirect immunofluorescent staining with Leu-11a or 3G8, respectively, as described in Materials and Methods.

[2]Results are expressed as mode values for fluorescence of fluorescin isothiocyanate-stained cells obtained from one experiment.

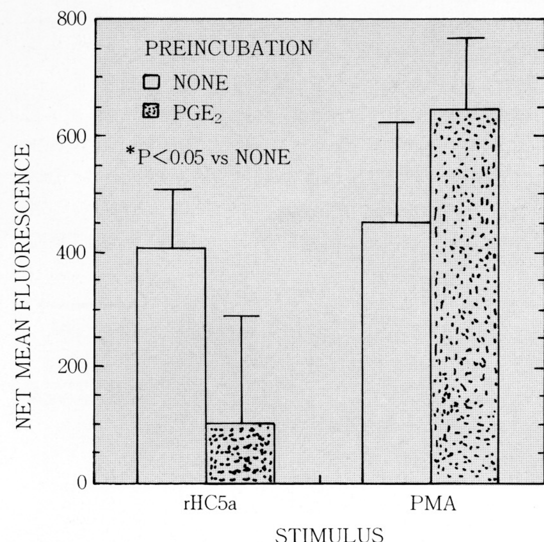

Fig. 3. Effects of PGE_2 upon the stimulated expression of CD11b by PMN. Data from five separate experiments are expressed as net mean(±SEM) fluorescence of mode values for PMN stained with phycoerythrin-conjugated anti-CD-11b monoclonal antibody. Net values were calculated by subtracting the mean fluorescnce obtained for cells incubated at 37℃(with or without PGE_2) from the mean fluorescence obtained from cells exposed to stimuli(see legend).

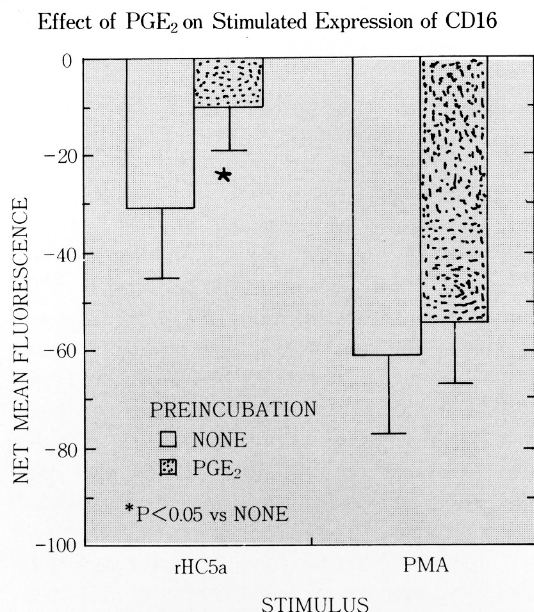

Fig. 4. Effect of PGE_2 upon the stimulated expression of CD16 by PMN. Data from five separate experiments are expressed as net mean (±SEM) fluorescence of mode values for PMN stained with fluorescein isothiocyanate-conjugated anti-CD16 monoclonal antibody. Net values were calculated by subtracting the mean fluorescence obtained for cells incubated at 37℃ (with or without PGE_2) from the mean fluorescence obtained from cells exposed to stimuli(see legend).

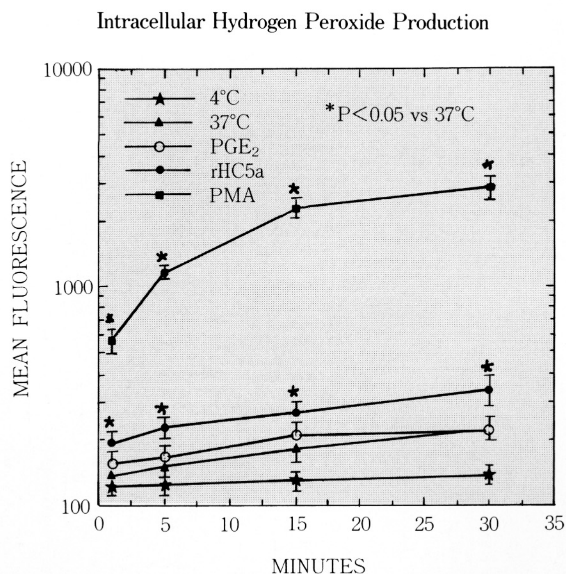

Fig. 5. Time course of intracellular hydrogen peroxide production by PMN. Following pre-incubation with 2′,7′-dichlorofluorescin diacetate, PMN were exposed to conditions(see legend) for the time indicated. Data from three separate experiments are expressed as mean fluorescence (±SEM) of PMN intracellular levels of 2′,7′-dichlorofluorescein.

Briefly, cells were incubated with 5.0 μM 2′,7′-dichoplorofluorescin diacetate and then stimuli were added to the incubation mixture. Reactions were terminated by addition of 5.0 mM NaN_3. The basal level of H_2O_2 production was obtained by maintaining cells at 4℃ throughout the experiment and is compared to stimulated production. Incubation at 37℃ alone produced a significant increase in H_2O_2 production. PGE_2(10^{-7} M) did not increase the amount of H_2O_2 as compared to incubation at 37℃ and was incapable of stimulating oxidative activity. rHC5a was a mild stimulus for H_2O_2 production and significantly increased the intracellular levels above that from cells maintained at 37℃. In contrast PMA produced a profound increase in H_2O_2 as compared to any other conditions. The stimulated H_2O_2 production reached a plateau level by 30 min. and was not significantly different for any stimuli at 60 min(data not shown). Control incubations with the diluent of the soluble stimuli(ethanol) at identical concentrations to those in the experiments did not show any significant changes in H_2O_2 production(data not shown).

To determine the effect of PGE_2 upon stimulated H_2O_2 production, we pre-incubated PMN previously pre-load with 5.0 μM 2′,7′-dichlorofluorescin diacetate in the presence or absence of 10^{-7} M PGE_2 at 37℃ for 15 min and then exposed the cells to stimuli for an additional 15 min at 37℃. Prolongation of the pre-incubation(30-60 min) with PGE_2 did not show any increase in its observed effect(data not shown). Fig. 6 displays the net intracellular H_2O_2 production by PMN as mean cell fluorescence for 2′,7′-dichlorofluorescin. The net stimulated H_2O_2 production following exposure to rHC5a was suppressed to a significant degree by PGE_2. In contrast, net production stimulated by PMA was not affected.

DISCUSSION

This study demonstrates that PGE_2 is capable of selectively suppressing some functional and phenoty-

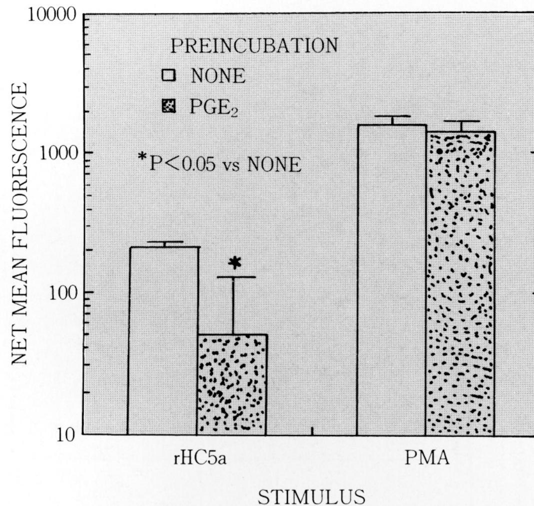

Fig. 6. Effect of PGE_2 upon the stimulated intracellular hydrogen peroxide production by PMN. Data from 5 separate experiments are expressed as net mean(±SEM) fluorescence of PMN intracellular levels of 2′,7′-dichlorofluorescein. Net values were calculated by subtracting the mean fluorescence obtained for cells incubated at 37℃ (with or without PGE_2)from the mean fluorescence obtained from cells exposed to stimuli(see legend).

pic responses of human PMN. When exposed to the membrane receptor stimulus C5a, PMN produced less intracellular H_2O_2 when pre-treated with 10^{-7} M PGE_2. The same concentrations of PGE_2 also interfered with C5a-induced changes in CD11b/CD16 expression. PGE_2 prevented the increased expression of CD11b and the decreased expression of CD16 that normally follows C5a stimulation of PMN.

In contrast to the observations with C5a, PMA-induced oxidation and intracellular H_2O_2 was not affected by PGE_2. Concomitantly, significant alterations of CD11b and CD16 expression were also not affected by pretreatment with PGE_2.

Others have reported that PMN respond to soluble stimuli by increasing the number of CR3(CD11b) on their surface(Berger et al., 1984 ; Kishimoto et al., 1989). It is believed that this occurs following fusion of lysosomal membranes with the plasma membrane, thus mobilizing an internal store of CR3 to the PMN surface(O'Shea et al., 1985). PMN regulated in this fashion are believed to be functionally activated("primed") for adherence or binding to the ligand may be altered with time. Despite high levels of CR3 on the surface, prolonged incubation of PMN with PMA for 65 min causes a 12-to 20-fold decrease("deactivation") in the ability of PMN to bind erythrocytes coated with C3bi(Wright and Meyer, 1986).

The fate of CD16 on human PMN has been studied in some detail following stimulation of PMN with fMLP. It was shown that stimulation of PMN caused the CD16 antigen to be released from the surface of the PMN into the supernatant(Huizinga et al., 1989a). The clinical significance of this observation has not been determined. The role of different FcgRs in PMN stimulation and phagocytosis is complex and may involve cross-linking of different types of FcgRs. Two FcgRs found on PMN, CD16 and FcgR II(CDw32)appear to mediate binding and internalization of immune complexes containing IgG(Huizinga et al ., 1989b). Of the two, only CD16 appears to be linked to phosphatidylinositol(Selvaraj et al., 1988 ; Darby et al., 1990). However, generation of oxidative burst activity by PMN stimulated with immune complexes may involve interaction of both CD16 and CDw32 with ligand(Huizinga et al., 1989b).

We used the assay for intracellular production of H_2O_2 to measure oxidative burst activity largely because of its high sensitivity. As originally described, this assay can detect changes in H_2O_2 within individual cells on the order of 10^{-18} molar(Bass et al., 1986). This assay relies upon the fact that the florochrome(2′,7′-dichlorofluorescin) is trapped within the cell following uptake, and the fluorescent material(2′,7′-dichlorofluorescin) is unable to leak out. Thus, total cell fluorescence indirectly reflects intracellular hydrogen peroxide production, Recently, similar methodology was used to measure H_2O_2 in PMN vacuoles(Ryan et al., 1990). Our results indicate that membrane stimulated oxidative burst activity was selectively inhibited by PGE_2.

Prior studies of trauma patients have shown that levels of PGE_2 in plasma following blunt injury were in the range of 1.0-3.0 ng/ml(Niennemann et al., 1984 ; Faist et al.,1987). We exposed PMN to a similar concentration of PGE_2 for our inhibition studies. The sources of PGE_2 in trauma patients has not been ascertained, although evidence indicates that compared to normal cells, monocytes taken from injured patients elicit more PGE_2 when stimulated with muramyl dipeptide(Miller-graziano et al., 1988). Evidence also indicates that PGE_2 may directly suppress

monocyte functions(Faist et al., 1987).

A host of studies have documented an immunosuppressive role for PGE_2. Studies examining proliferation of lymphocytes induced by interleukin-2 have shown interference by PGE_2(Faist et al., 1987). PGE_2 also interfered with cell-mediated immunity in thermally injured mice(Freeman and Shelby, 1988), lowered Ia expression on monocytes(Snyder et al., 1982), and directly suppressed antigen presentation in vitro(Stephan et al., 1988). One study for in vivo on the effects of PGE_2 suggests that infusion of this agent failed to impair cell-mediated immunity(Waymack and Yurt, 1988). This contrast between in vitro and in vivo effects has not been resolved.

Other investigators have demonstrated a direct inhibitory effect of PGE_2 on certain PMN responses. Ward, et. al., observed a suppressive effect of prostaglandins on superoxide production using rat PMN and soluble stimuli(Ward et al., 1984). In a study of PMN from atopic and non-atopic donors, PGE_2 was less capable than PGD_2 in suppressing superoxide anion production by either group of PMN stimulated by fMLP. In contrast, PMA-stimulated superoxide anion production was not affected by the prostaglandins(Styrt et al., 1988). Chemotactic activity of PMN in response to leukotriene B4 is enhanced by PGE_2(Michel et al., 1987). Our studies are consistent with these results in that intracellular hydrogen peroxide production stimulated by a chemotactic agent was suppressed by PGE_2, whereas, PMA stimulation was unaffected.

We were unable to document any studies that examined the effect of PGE_2 on either CD16 or CD11b expression. One recent report indicates that phenotypic expression of both antigens is widely variable amongst thermally-injured individuals, however, decreased expression of both antigens concomitantly, was associated with infection and sepsis(Babcock et al., 1990). In this study, decreased expression of CD16 can be explained by PMN activation in vivo.

The clinical consequences of depressed CD11b expression remain to be clarified, however, in light of other studies that indicate increased levels of CD11b following activation by known physiologic stimuli(i.e. C5a or immune complexes or following burn injury-(Moore et al., 1986). The additional clinical impact of PGE_2 upon CD11b/CD16 expression will also require further study.

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