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Effect of leukotriene B_4 and prostaglandin E_2 on the adhesion of lymphocytes to endothelial cells

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SUMMARY

The arachidonic acid metabolites leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) may play an important role in inflammation. It is not known whether these mediators influence the binding of lymphocytes to endothelial cells, a process which is important in the extravasation of lymphocytes in inflammatory states. In the present investigation, the effect of LTB_4 and PGE_2 on the binding interaction between lymphocytes and endothelial cells was examined using a centrifugation cell binding assay. Although LTB_4 elicited an aggregation response on human polymorphonuclear leucocytes (PMNL) and enhanced their binding to endothelial cells it had no effect on lymphocyte binding. By contrast, PGE_2 caused a dose-dependent inhibition of lymphocyte binding to endothelial cells. The inhibitory effect of PGE_2 had a rapid onset but was exhibited only when PGE_2 was present continuously during the cell binding assay. Although the mechanism by which PGE_2 acts is not clear, it may provide a negative feedback mechanism in regulating the influx of lymphocytes into inflammatory sites.

Keywords lymphocytes endothelial cells binding leukotriene B_4 prostaglandin E_2

INTRODUCTION

Lymphocytes are highly mobile cells which continuously recirculate between the vascular compartment and lymphoid tissues via specialized post-capillary venules known as high endothelial venules (Gowans & Knight, 1964). Morphologically and functionally similar post-capillary venules are also present in chronically inflamed tissues (Freemont & Ford, 1985).

The adherence of lymphocytes to endothelium is one of the important initial events in the migration of lymphocytes through the vascular wall. It has been proposed that peptide cytokines such as interleukin-1 (IL-1), tumour necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) are important in the migration of lymphocytes as they enhance the binding of lymphocytes to endothelial cells (Cavender *et al.*, 1986; Cavender, Saegusa & Ziff, 1987; Yu *et al.*, 1985; Masuyama, Minato & Kano, 1986). The cytokines also induce the expression of specific surface antigens on endothelial cells and these may assist in the recruitment of lymphocytes into tissues (Pober *et al.*, 1983, 1986a).

Little is known about the role of lipid mediators of inflammation on the adhesive interaction of lymphocytes and endothelial cells. The two principal lipid mediators produced in leucocytes are leukotriene B_4 (LTB_4) and prostaglandin E_2 (PGE_2) which are derived from arachidonic acid via the 5-lipoxygenase and cyclooxygenase pathways, respectively (Needleman *et al.*, 1986). LTB_4 is a potent chemoattractant for polymorphonuclear leucocytes (PMNL) *in vitro* (Ford-Hutchinson *et al.*, 1980) and causes their migration into perivascular sites when applied topically to the skin (Camp *et al.*, 1984; Winklemann *et al.*, 1986). *In vitro*, LTB_4 enhances the binding of PMNL to endothelial cells (Hoover *et al.*, 1984; Gimbrone, Brock & Schafer, 1984). It has recently been reported that LTB_4 also increases the binding of lymphocytes to rat endothelial cells *in vitro* (Renkonen *et al.*, 1988). PGE_2, on the other hand, exerts inhibitory effects on lymphocyte functions. It inhibits lymphocyte chemotaxis *in vitro* (Van Epps, 1981) and causes cell shutdown in lymph nodes (Hopkins, McConnell & Pearson, 1981).

In the work reported here, we have used the centrifugation cell binding assay of McClay *et al.* (1981), with minor modifications, to examine the effect of LTB_4 and PGE_2 on lymphocyte–endothelial cell binding interactions. This reproducible technique has been previously applied to a study of the adherence of PMNL to endothelial cells (Charo *et al.*, 1985, 1986). Using this technique, we have demonstrated that whereas LTB_4 enhances the adhesion of PMNL to endothelial cells it has no effect on lymphocyte binding to endothelial cells. In contrast, PGE_2 causes a rapid onset, dose-dependent inhibition of lymphocyte binding.

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MATERIALS AND METHODS

Endothelial cell tissue culture

Endothelial cells were obtained from human umbilical veins by collagenase digestion as previously described (Jaffe *et al.*, 1973). Cells were maintained in growth medium consisting of M199 supplemented with 20% heat-inactivated fetal calf serum (FCS) (Commonwealth Serum Laboratories, Victoria, Australia), 15 mM $NaHCO_3$, 20 mM HEPES buffer, 100 U/mL penicillin, 100 μg/ml streptomycin, 100 μg/ml bovine lung heparin (Sigma Chemical Company, St Louis, MO) and 50 μg/ml endothelial cell growth supplement (Collaborative Research, Bedford, MA). Cells in the monolayer were confirmed to be endothelial in origin by immunoperoxidase staining with rabbit anti-human factor VIII-related antigen serum (Dakopatts, Glostrup, Denmark). At confluence, cells were detached with 0·05% trypsin (Commonwealth Serum Laboratories) and 0·02% EDTA (Ajax Chemicals, Sydney, Australia). Only endothelial cells of passages two to four were used.

Preparation of LTB_4 and PGE_2

LTB_4 was prepared from rat PMNL according to the method of Forrest, Zammit & Brooks (1988). Rat PMNL were obtained from the peritoneal cavities of animals 4 h after the injection of 20 ml of a 5% peptone (Oxoid, Hampshire, UK) solution in phosphate-buffered saline. The cells were washed in Ca^{++}- and Mg^{++}-free HBSS (Sigma) and then resuspended in complete HBSS containing 0·25% bovine serum albumin (BSA, Sigma). The cells were incubated with arachidonic acid (10 μg/ml, Sigma) and calcium ionophore A23187 (1 μg/ml, Sigma) for 15 min at 37°C. The cell suspension was immediately centrifuged at 2500 ***g*** for 15 min and LTB_4 was extracted from the supernatant using a C-18 solid phase extraction column (Baker Chemical Company, Phillipsburg, NJ). This crude extract was further purified by reverse-phase high performance liquid chromatography using an Altex Ultrasil ODS column (Beckman Instruments, Altex Division, San Ramon, CA). LTB_4 was identified and measured by comparison with the elution time and peak height of synthetic LTB_4 (a generous gift from Dr J. Rokach, Merck, Frosst, Canada). The biological activity of the LTB_4 preparation was tested by performing an aggregation assay on both rat and human PMNL according to a previously described method (Ringertz *et al.*, 1982). PGE_2 was obtained commercially from Sigma and reconstituted in 100% ethanol.

Isolation of normal human lymphocytes

Mononuclear cells from normal peripheral blood were separated by centrifugation on Ficoll–Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). Lymphocytes were obtained by counter-flow centrifugal elutriation according to the method of Hopper *et al.* (1987). Mononuclear cells were resuspended in elutriation medium consisting of Ca^{++}- and Mg^{++}-free HBSS containing 0·4% BSA, 0·38% trisodium citrate (Ajax Chemicals), 15 mM $NaHCO_3$ and adjusted to pH 7·4. Elutriation was performed using the Beckman JE-6B elutriator rotor with a standard chamber (Beckman Instruments). Flow rates of elutriation medium were from 8·0 to 22·5 ml/min with the rotor running at 540 ***g***. Fractions 2 and 3, consisting of mainly lymphocytes, were pooled. This preparation of cells contained less than 3% $63D3^+$ cells (monocytes/macrophages) as determined by flow cytometric analysis.

^{51}Cr labelling of lymphocytes

Lymphocytes were resuspended in M199 supplement with 20% FCS. $Na_2{}^{51}CrO_4$ was added at a level of 10μCi/10^6 cells giving a final radioactivity of 500 μCi/ml. The cells were incubated at 37°C for 60 min, then washed twice with 10 ml M199 to remove soluble ^{51}Cr. Residual ^{51}Cr and dead cells were removed by centrifuging the labelled cells through 4 ml of FCS.

Lymphocyte–endothelial cell binding assay

With minor modifications, lymphocyte adherence to endothelial cells was assayed by the centrifugation cell binding method of McClay *et al.* (1981). Endothelial cells at confluence were removed from culture flasks with trypsin and EDTA, centrifuged and resuspended in M199 with 20% FCS at a concentration of 2×10^5 cells/ml. Aliquots of 0·2 ml were then plated onto 96-well plates composed of strips of flat-bottomed wells (Dynatech Laboratories, Chantilly, VA) precoated with human fibronectin (10 μg/ml, Collaborative Research). Endothelial cells were cultured overnight after which time confluence was usually achieved. ^{51}Cr-labelled lymphocytes were resuspended at a concentration of 1×10^6 cells/ml in assay medium (M199 + 10% FCS). Aliquots of 0·2 ml were then added to each well and the 96-well plates were spun at 50 ***g*** for 1 min in a centrifuge equipped with plate carriers (H.I. Clements, North Ryde, NSW, Australia). Lymphocytes and endothelial cells were allowed to interact in a humidified incubator of 5% CO_2 and 95% air for 10 min at 37°C. Excess warm assay medium was then added to each well to create a positive meniscus and the plates were sealed with adhesive plastic sealers avoiding trapping air bubbles. The plates were inverted and centrifuged at 400 ***g*** for 10 min at 22°C. While still inverted, the plates were snap-frozen in ethanol/dry ice mixture. The bottoms of the wells were cut off using an animal toenail cutter and the extent of lymphocyte binding was determined by gamma counting. The percentage of lymphocytes bound was calculated as follows:

$$\frac{\text{ct/min bound lymphocytes}}{\text{ct/min lymphocytes added}} \times 100\%$$

Treatment of lymphocytes and/or endothelial cells

Lymphocytes and endothelial cells were either pretreated or coincubated with LTB_4 or PGE_2 at 37°C for up to 60 min. Pretreated lymphocytes were washed twice with assay medium before the binding assay. Pretreated endothelial cells in 96-well plates were washed once with 0·2 ml assay medium before lymphocytes were added.

Statistical analysis

Results were analysed by one-way multiple analysis of variance.

RESULTS

Validation of lymphocyte–endothelial cell centrifugation binding assay using cytokines and mitogens

Treatment of endothelial cells with IFN-γ, IL-1 or phorbol myristate acetate (PMA) enhances lymphocyte binding (Yu *et al.*, 1985; Cavender *et al.*, 1986; Haskard, Cavender & Ziff, 1986). Treatment of lymphocytes with phorbol dibutyrate $P(Bu)_2$ increases their adherence to endothelial cells (Haskard *et al.*, 1986). We used recombinant IFN-γ (a generous gift from Dr G. Adolf, Ernst-Boehringer Institute fur Arzneimittelforschung,

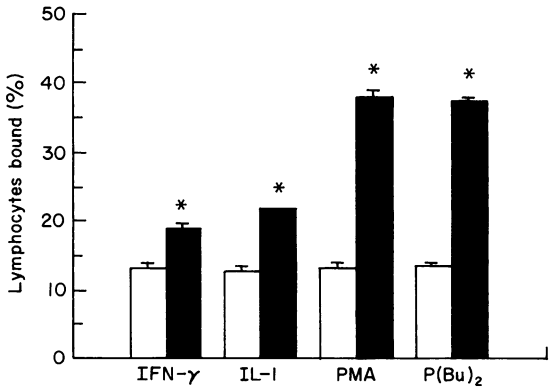

Fig. 1. Evaluation of lymphocyte–endothelial cell centrifugation binding assay using known positive controls. Endothelial cells were pretreated with IFN-γ (250 U/ml, 20 h), purified IL-1 (5 U/ml, 5 h), PMA (100 ng/ml, 1 h) or medium control (□), and washed with 0·2 ml assay medium before the addition of untreated lymphocytes. $P(Bu)_2$ (50 ng/ml) or medium was used to pretreat lymphocytes for 15 min and washed twice with assay medium before overlaying on to untreated endothelial cells. Mean ± s.d. of triplicate determinations of a representative experiment. $*P < 0{\cdot}001$.

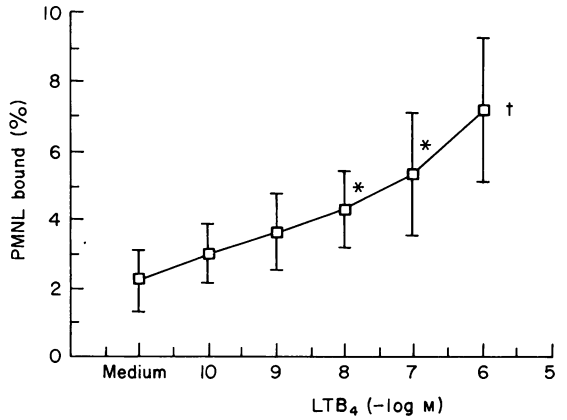

Fig. 2. Dose-dependent enhancement effect of LTB_4 on PMNL–endothelial cell binding. PMNL and endothelial cells were coincubated with assay medium or LTB_4 for 10 min at 37°C before non-adherent PMNL were removed by centrifugation. Mean ± s.d. of means of four separate experiments each conducted in triplicate. $*P < 0{\cdot}05$; $\dagger P < 0{\cdot}005$.

Vienna, Austria), purified IL-1 (Cistron Biotechnology, Pine Brook, NJ), PMA (Sigma) and $P(Bu)_2$ (Sigma) as positive controls to evaluate the centrifugation technique on lymphocyte–endothelial cell binding. Figure 1 shows that all positive controls tested enhanced lymphocyte-EC binding. Good reproducibility between replicates was consistently achieved with a coefficient of variation of less than 10%.

Biological activity of biosynthesized LTB_4

LTB_4 obtained from rat PMNL caused both rat and human PMNL to aggregate (data not shown). The same LTB_4 preparation also enhanced the binding of human PMNL to endothelial cells in a dose-dependent fashion, as shown in Fig. 2. The enhancement was statistically significant at LTB_4 concentrations $\geq 10^{-8}$M. Basal binding of PMNL to endothelial cells in the presence of medium alone was substantially lower (2–4%) than that observed with lymphocytes (12–30%), which is consistent with a previous report (Pober *et al.*, 1986b).

Table 1. Lack of effect of leukotriene B_4 (LTB_4) on lymphocyte–endothelial cell binding

	Lymphocyte–endothelial cell binding (%)				
		LTB_4 concentration			
Donor	Medium control	10^{-12}M	10^{-10}M	10^{-8}M	10^{-6}M
1(a)	17·0 ± 1·4	16·6 ± 1·4	16·5 ± 1·1	16·2 ± 2·1	18·1 ± 0·4
2(a)	29·4 ± 2·9	29·3 ± 2·0	29·3 ± 2·9	28·5 ± 0·9	28·9 ± 1·8
3(a)	25·8 ± 0·4	25·7 ± 0·9	26·0 ± 0·6	26·7 ± 1·6	25·7 ± 0·5
4(b)	15·2 ± 0·9	19·2 ± 2·2	17·9 ± 0·6	18·2 ± 1·4	16·5 ± 1·0
5(b)	19·9 ± 1·3	22·7 ± 0·6	24·2 ± 2·3	23·3 ± 0·7	24·0 ± 1·2
6(b)	16·9 ± 3·0	16·6 ± 0·9	17·7 ± 0·7	19·4 ± 0·6	19·1 ± 0·6
7(c)	23·3 ± 0·3	23·8 ± 1·3	25·0 ± 1·4	23·2 ± 1·0	22·7 ± 1·8
8(c)	20·4 ± 0·2	22·1 ± 2·6	21·2 ± 0·4	21·5 ± 0·3	21·8 ± 0·7
9(c)	24·0 ± 1·5	23·2 ± 0·7	23·7 ± 0·4	23·4 ± 0·2	23·4 ± 1·3

Cells were incubated with assay medium or LTB_4 for 60 min at 37°C. (a) Endothelial cells alone; (b) lymphocytes alone; and (c) endothelial cells and lymphocytes together. Data are expressed as mean ± s.d. of triplicate determinations. Different individual donors exhibit considerable differences in baseline binding.

Effect of LTB_4 on lymphocyte–endothelial cell binding

Pretreatment of either endothelial cells or lymphocytes alone with LTB_4 at any dose (10^{-12}–10^{-6} M) for up to 60 min had no effect on lymphocyte–endothelial cell binding. Similarly, coincubation of lymphocytes and endothelial cells with LTB_4 had no effect on binding, even at LTB_4 concentrations of 10^{-6}M (Table 1).

Effect of PGE_2 on lymphocyte–endothelial cell binding

To determine the effect of PGE_2 on lymphocyte binding, lymphocytes and endothelial cells were coincubated with 10^{-6} M PGE_2 for various periods of time before the non-adherent lymphocytes were removed by centrifugation. As shown in Fig. 3, the inhibitory effect of PGE_2 had a rapid onset (5 min) and reached a maximum after 20 min. Although PGE_2 was reconstituted in ethanol, controls containing equivalent amounts of ethanol did not show any inhibitory effect (data not shown). Furthermore, the presence of ethanol did not affect the viability of either lymphocytes or endothelial cells during the 60 min incubation (viability > 96% by trypan blue exclusion test). The inhibitory effect of PGE_2 was dose-dependent with the greatest inhibition occurring at 10^{-6} M (Fig. 4). The results with PGE_2 concentrations greater than 10^{-6} M could not be meaningfully interpreted as the equivalent amounts of ethanol used as the control caused disruption of endothelial cells monolayer over a 60-min incubation period.

Effect of prostacyclin (PGI_2) on lymphocyte–endothelial cell binding

In order to examine whether PGI_2 has the same effect as PGE_2 on lymphocyte binding, a stable PGI_2 analogue, iloprost

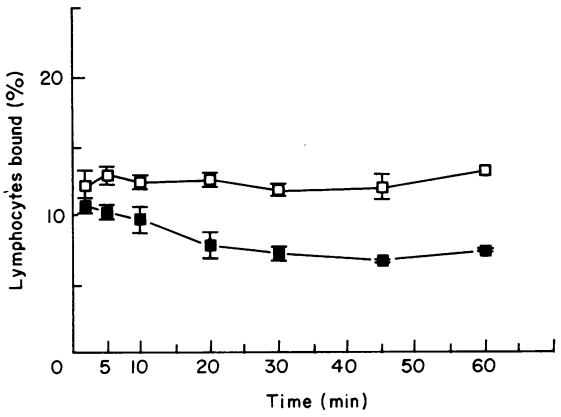

Fig. 3. Kinetics of PGE_2 inhibition on lymphocyte–endothelial cell binding. Significant inhibition was observed shortly (5 min) after the addition of PGE_2 and maximum inhibition was reached after 20 min. The difference between PGE_2-treated wells and control wells was significant ($P < 0{\cdot}001$) from 5 min onwards and remained constant from 20 min to 60 min incubation. Results are from a representative experiment and expressed as mean ± s.d. of triplicate determinations. □, Medium; ■, 10^{-6} M PGE_2.

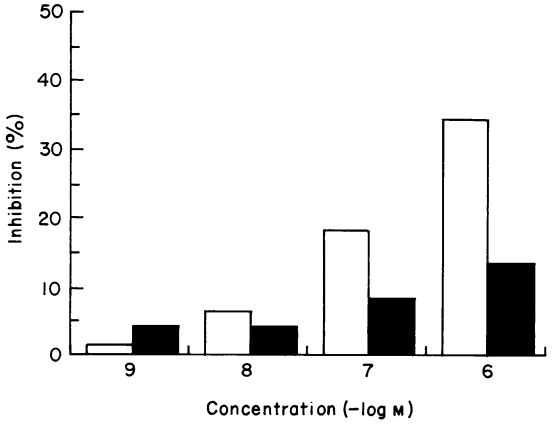

Fig. 4. Dose-dependent inhibition of lymphocyte–endothelial cell binding by PGE_2 (□) and iloprost (■). Lymphocytes and endothelial cells were co-incubated with either PGE_2 or iloprost for 60 min at 37°C. Results, from a representative experiment of at least three other experiments, are expressed as the percentage inhibition calculated by the formula:

$$\left(1-\frac{\text{mean \% binding in PGE}_2\text{/iloprost wells}}{\text{mean\% binding in control wells}}\right)\times 100\%$$

(ZK36374, generously donated by Schering, Berlin, FRG), was used. The iloprost was biologically active as it inhibited platelet-activating factor induced aggregation of washed rabbit platelets (data not shown). However, it had only minor inhibitory effects on lymphocyte–endothelial cell binding by comparison with the effects of PGE_2 used at equivalent concentrations (Fig. 4).

Inhibition of lymphocyte–endothelial cell binding requires presence of PGE$_2$

In an effort to determine whether the inhibitory effect of PGE_2 was exerted principally on the lymphocytes or the endothelial cells, the two types of cells were pretreated separately with PGE_2 for 60 min at 37°C and then washed with assay medium before the binding assay. However, the inhibitory effect of PGE_2 was totally abolished when PGE_2 was not present during the binding assay (data not shown).

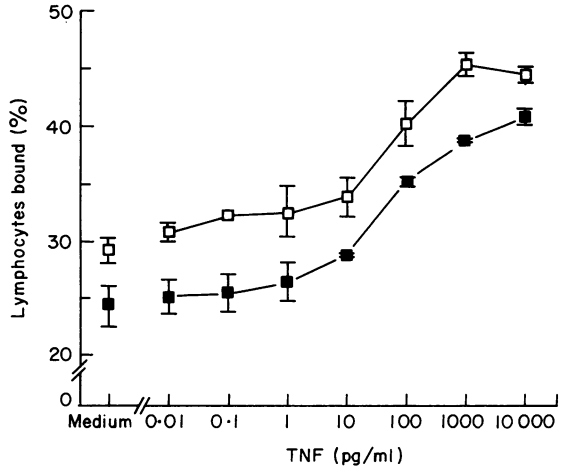

Fig. 5. Effect of PGE_2 on lymphocyte binding to TNF-α-treated endothelial cells. Endothelial cells were pretreated with assay medium or recombinant TNF-α for 4 h at 37°C before lymphocytes were added. Lymphocytes and endothelial cells were then co-incubated with 10^{-6} M PGE_2 (■) or assay medium containing equivalent concentrations of ethanol (□) for 60 min at 37°C. Results are from a representative experiment and expressed as mean ± s.d. of triplicate determinations.

Effect of PGE$_2$ on lymphocyte binding to cytokine-treated endothelial cells

We evaluated whether PGE_2 could inhibit the enhanced lymphocyte adhesion to endothelial cells pretreated with cytokines. Endothelial cells were treated with human recombinant TNF-α (Genzyme Corporation, Boston, MA) for 4 h at 37°C prior to the lymphocyte–endothelial cell binding assay. TNF-α was removed and endothelial cells were washed once with 0·2 ml assay medium before lymphocytes were overlaid. Lymphocytes and endothelial cells were then incubated either with 10^{-6} M PGE_2 or assay medium containing equivalent amounts of ethanol for 60 min at 37°C. TNF-α induced a dose-dependent enhancement of lymphocyte binding to endothelial cells (Fig. 5). Addition of 10^{-6} M PGE_2 resulted in inhibition of lymphocyte binding, but the effect was constant independent of the TNF-α concentration used. Treatment with 10^{-6} M PGE_2 thus failed to abrogate the augmented lymphocyte adhesion observed with TNF-α concentrations of $\geqslant 10$ pg/ml (Fig. 5).

DISCUSSION

In the present investigation, we have used a centrifugation cell binding assay (McClay *et al.*, 1981), with minor modifications, to study the effect of LTB_4 and PGE_2 on lymphocyte–endothelial cell binding interaction. This centrifugation assay provides good reproducibility between replicates with coefficient of variation consistently less than 10%. The assay has allowed us to confirm previous observations that pretreatment of endothelial cells with IL-1, IFN-γ or PMA enhances the adhesion of lymphocytes (Yu *et al.*, 1985; Cavender *et al.*, 1986; Masuyama *et al.*, 1986; Haskard *et al.*, 1986) while pretreatment of

lymphocytes with $P(Bu)_2$ increases their ability to bind to endothelial cells (Haskard *et al.*, 1986).

In this study, LTB_4 had no effect on lymphocyte–endothelial cell binding at any concentration tested. The lack of effect was not due to a lack of biological activity, since the same LTB_4 preparation caused aggregation of both rat and human PMNL and also enhanced the adhesion of human PMNL to endothelial cell monolayers. Our observations are at variance with those recently reported by Renkonen *et al.* (1988) of increased lymphocyte binding to endothelial cells following LTB_4 treatment. However, those investigators used a rodent model and this may account for the disparity.

Most investigations of the effect of mitogens (e.g. PMA, $P(Bu)_2$, bacterial lipopolysaccharide) and cytokines (IL-1, TNF-α, IFN-γ) on lymphocyte–endothelial cell binding have reported enhanced binding (Yu *et al.*, 1985, 1986; Haskard *et al.*, 1986; Masuyama *et al.*, 1986; Cavender *et al.*, 1986, 1987). By contrast, we have demonstrated that PGE_2 causes a dose-dependent inhibition of lymphocyte binding. Inhibition was observed with 10^{-8} M PGE_2 and reached a maximum of 30% reduction of basal binding at 10^{-6}) (Fig. 4), the highest concentration used in this study. However, we were unable to determine whether the lymphocytes or the endothelial cells were the target of PGE_2 effect, due to the rapid loss of inhibition when PGE_2 was removed. The failure of PGE_2 to abrogate the augmented lymphocyte binding observed after treatment of endothelial cells with TNF-α (Fig. 5) suggests that lymphocytes rather than endothelial cells are the target of PGE_2. PGE_2 inhibition cannot be explained by a loss of lymphocyte viability. The reason for partial inhibition (30%) is unclear, although possible explanations include an effect on only subset(s) of lymphocytes present in the overlaid population. Experiments with lymphocyte subpopulations may help to delineate which lymphocyte subset(s) is responsive to PGE_2.

The mechanism by which PGE_2 inhibits lymphocyte binding is unclear. Its rapid onset suggests that it causes changes in cell membrane properties rather than acting through new protein synthesis as occurs with cytokine stimulation of endothelial cells where enhanced binding is observed only hours after the application of cytokines (Yu *et al.*, 1985; Masuyama *et al.*, 1986; Cavender *et al.*, 1986, 1987). PGE_2 enhances the production of cAMP in lymphocytes and this molecule plays a second messenger role in PGE_2-mediated responses (Goodwin, Bromberg & Messner, 1981). Similar or more potent inhibitory effects on lymphocyte–endothelial cell binding might be anticipated with PGI_2 compared with PGE_2 because it is a more potent stimulator of adenylate cyclase activity, at least in platelets (Gorman, Bunting & Miller, 1977), than PGE_2. However, iloprost (the stable PGI_2 analogue) had only a minor inhibitory effect on lymphocyte–endothelial cell binding compared with PGE_2 used at equimolar concentrations, although it inhibited platelet aggregation induced by platelet-activating factor. The lack of effect of iloprost may be due to the absence of PGI_2/iloprost receptors on lymphocytes, whereas the presence of PGE_2 receptors on lymphocytes has previously been demonstrated (Goodwin *et al.*, 1979).

Results from our preliminary experiments (data not shown) support a role for cAMP as the second messenger in the inhibitory effect of PGE_2 on lymphocyte–endothelial cell binding. Dibutyryl-cAMP, a lipophilic analogue of cAMP, and forskolin, an activator of adenylate cyclase, both inhibited lymphocyte–endothelial cell binding. In addition, there is a dose-dependent increase of cAMP levels measured by radioimmunoassay in lymphocytes treated with either PGE_2 or forskolin (data not shown). The ability of PGE_2 to inhibit lymphocyte adhesion to endothelial cells as well as lymphocyte chemotaxis (Van Epps, 1981) may indicate its role in the regulation of lymphocyte extravasation into inflammatory sites.

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REFERENCES

CAMP, R., JONES, R.R., BRAIN, S., WOOLLARD, P. & GREAVES, M. (1984) Production of intraepidermal microabscesses by topical application of leukotriene B_4. *J. clin. Invest.* **82,** 202.

CAVENDER, D.E., SAEGUSA, Y. & ZIFF, M. (1987) Stimulation of endothelial cell binding of lymphocytes by tumour necrosis factor. *J. Immunol.* **139,** 1855.

CAVENDER, D.E., HASKARD, D.O., JOSEPH, B. & ZIFF, M. (1986) Interleukin 1 increases the binding of human B and T lymphocytes to endothelial cell monolayers. *J. Immunol.* **136,** 203.

CHARO, I.F., YUEN, C. & GOLDSTEIN, I.M. (1985) Adherence of human polymorphonuclear leukocytes to endothelial monolayers: effects of temperature, divalent cations, and chemotactic factors on the strength of adherence measured with a new centrifugation assay. *Blood,* **65,** 473.

CHARO, I.F., YUEN, C., PEREZ, D. & GOLDSTEIN, I.M. (1986) Chemotactic peptides modulate adherence of human polymorphonuclear leukocytes to monolayers of cultured endothelial cells. *J. Immunol.* **136,** 3412.

FORD-HUTCHINSON, A.W., BRAY, M.A., DOIG, M.V., SHIPLEY, M.E. & SMITH, M.J.H. (1980) Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature,* **286,** 264.

FORREST, M.J., ZAMMIT, V. & BROOKS, P.M. (1988) Inhibition of leukotriene B_4 synthesis by BW755c does not reduce polymorphonuclear leukocyte (PMNL) accumulation induced by monosodium urate crystals. *Ann. rheum. Dis.* **47,** 241.

FREEMONT, A.J. & FORD, W.L. (1985) Functional and morphological changes in postcapillary venules in relation to lymphocyte infiltration into BCG-induced granulamata in rat skin. *J. Pathol.* **147,** 1.

GIMBRONE, M.A. JR, BROCK, A.F. & SCHAFER, A.I. (1984) Leukotriene B_4 stimulates polymorphonuclear leukocyte adhesion to cultured vascular endothelial cells. *J. clin. Invest.* **74,** 1552.

GOODWIN, J.S., BROMBERG, S. & MESSNER, R.P. (1981) Studies on the cyclic AMP response to prostaglandins in human lymphocytes. *Cell Immunol.* **60,** 298.

GOODWIN, J.S., WILK, A., LEWIS, M., BANKHURST, A.D. & WILLIAMS, R.C. JR (1979) High-affinity binding sites for prostaglandin E on human lymphocytes. *Cell Immunol.* **43,** 150.

GORMAN, R.R., BUNTING, S. & MILLER, O.V. (1977) Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins*, **13,** 377.

GOWANS, J.L. & KNIGHT (1964) The route of recirculation of lymphocytes in the rat. *Proc. R. Soc. Lond. [Biol.]* **159,** 257.

HASKARD, D., CAVENDER, D. & ZIFF, M. (1986) Phorbol ester-stimulated T lymphocytes show enhanced adhesion to human endothelial cell monolayers. *J. Immunol.* **137,** 1429.

HOOVER, R.L., KARNOVSKY, M.J., AUSTEN, K.F., COREY, E.J. & LEWIS, R.A. (1984) Leukotriene B_4 action on endothelium mediates augmented neutrophil/endothelial adhesion. *Proc. natl Acad. Sci. USA*, **81,** 2191.

HOPKINS, J., MCCONNELL, I. & PEARSON, J.D. (1981) Lymphocyte traffic through antigen-stimulated lymph nodes. II. Role of prostaglandin E_2 as a mediator of cell shutdown. *Immunology*, **42,** 225.

HOPPER, K.E., SEMLER, A.D., CHAPMAN, G.V. & DAVEY, R.A. (1986) Release of galactosyltransferase from human platelets and a subset of monocytes in culture. *Blood*, **68,** 167.

JAFFE, E.A., NACHMAN, R.L., BECKER, C.G. & MINICK, C.K. (1973) Culture of human endothelial cells derived from umbilical veins. *J. clin. Invest.* **52,** 2745.

MASUYAMA, J., MINATO, N. & KANO, S. (1986) Mechanisms of lymphocyte adhesion to human vascular endothelial cells in culture. T lymphocyte adhesion to endothelial cells through endothelial HLA-DR antigens induced by gamma interferon. *J. clin. Invest.* **77,** 1596.

MCCLAY, D.R., WESSEL, G.M. & MARCHASE, R.B. (1981) Intercellular recognition: quantitation of initial binding events. *Proc. natl Acad. Sci. USA*, **78,** 4975.

NEEDLEMAN, P., TURK, J., JALSCHIK, B.A., MORRISON, A.R. & LEFKOWITH, J.B. (1986) Arachidonic acid metabolism. *Annu. Rev. Biochem.* **55,** 69.

POBER, J.S., BEVILACQUA, M.P., MENDRICK, D.L., LAPIERRE, L.A., FIERS, W. & GIMBRONE, M.A. Jr (1986a) Two distinct monokines, interleukin 1 and tumour necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. *J. Immunol.* **136,** 1680.

POBER, J.S., GIMBRONE, M.A. JR, COTRAN, R.S., REES, C.S., BURAKOFF, S.J., FIERS, W. & AULT, K.A. (1983) Ia expression by vascular endothelium is inducible by activated T cells and by human gamma interferon. *J. exp. Med.* **157,** 1339.

POBER, J.S., GIMBRONE, M.A. JR, LAPIERRE, L.A. MENDRICK, D.L., FIERS, W., ROTHLEIN, R. & SPRINGER, T.A. (1986b) Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J. Immunol.* **137,** 1893.

RENKONEN, R., MATTILA, P., LESZCZYNSKI, D. & HAYRY, P. (1988) Leukotriene B_4 increases the lymphocyte binding of endothelial cells. *FEBS Lett.* **235,** 67.

RINGERTZ, B., PALMBLAD, J., RADMARK, O. & MALMSTEN, C. (1982)Leukotriene-induced neutrophil aggregation *in vitro*. *FEBS Lett.* **147,** 180.

VAN EPPS, D.E. (1981) Suppression of human lymphocyte migration by PGE_2. *Inflammation*, **5,** 81.

WINKLEMANN, R.K., CAMP, R., ENGLISH, J.S.C. & GREAVES, M.W. (1986) The perivascular cell populations in human skin after topical application of leukotriene B_4. *Acta dermatol. venerol.* **66,** 340.

YU, C.L., HASKARD, D.O., CAVENDER, D., JOHNSON, A.R. & ZIFF, M. (1985) Human gamma interferon increases the binding of T lymphocytes to endothelial cells. *Clin. exp. Immunol.* **62,** 554.

YU, C.L., HASKARD D., CAVENDER, D. & ZIFF, M. (1986) Effects of bacterial lipopolysaccharide on the binding of lymphocytes to endothelial cell monolayers. *J. Immunol.* **135,** 569.