Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 2397–2401, March 1991
Cell Biology

Adhesion protein GMP140 inhibits superoxide anion release by human neutrophils

(lectin/epidermal growth factor/complement-binding domain cell adhesion molecule/selectin/tumor necrosis factor α/inflammation/thrombosis)

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Communicated by J. F. A. P. Miller, December 17, 1990 (received for review August 3, 1990)

ABSTRACT **The respiratory burst of blood neutrophils has a critical role in the destruction of microorganisms and tissue damage in inflammation. Neutrophils adhere in a dose-dependent fashion to granule membrane protein 140 (GMP140), a member of the LEC-CAM (lectin/epidermal growth factor/complement-binding domain cell adhesion molecule) family of adhesion proteins when it is immobilized onto plastic surfaces. Adherence to GMP140 was associated with less superoxide anion generation than adherence to other surfaces, an effect that is especially remarkable after activation of neutrophils with tumor necrosis factor α, an agent that on other surfaces promotes adhesion and spreading. However, on GMP140 the cells fail to spread and instead remain rounded and refractile. Neutrophils adhering to GMP140 were also deficient in superoxide anion generation to formylmethionylleucylphenylalanine. Furthermore, fluid-phase GMP140 also inhibited the superoxide generation by neutrophils stimulated by tumor necrosis factor α. The effect of GMP140 was reversible by washing and was inhibited by anti-GMP140 Fab antibody. GMP140 appears to be a natural antiinflammatory molecule that may prevent the inappropriate activation of neutrophils in the circulation.**

The respiratory burst of blood neutrophils has a critical role in the destruction of microorganisms and tissue damage in inflammation. The prevention of inappropriate respiratory burst is therefore likely to be an important homeostatic mechanism. Granule membrane protein 140 (GMP140) is a 140-kDa integral membrane glycoprotein found in the α granules of platelets and in the Weibel–Palade bodies of endothelial cells (1–4). Upon activation of endothelial cells and platelets, GMP140 is rapidly redistributed to the plasma membrane and has been shown to mediate adhesion of activated platelets to monocytes (5) and neutrophils (5, 6) and adhesion between endothelial cells and neutrophils (7).

The cloning of GMP140 from human umbilical vein endothelial cells predicts a cysteine-rich protein with multiple domains, including calcium-dependent mammalian lectin, epidermal growth factor, and complement-binding domains. These domains are followed by a transmembrane sequence and a short cytoplasmic tail. Analysis of cDNA also suggests a soluble form with the transmembrane domain deleted (8). The structure of GMP140 is similar to two known proteins involved in leukocyte adhesion, the Mel-14 antigen (9) and ELAM-1 (10); these proteins are classified as LEC-CAMs (lectin/epidermal growth factor/complement-binding domain cell adhesion molecules) (11) or selectins (7). GMP140, as another member of the LEC-CAM family, ELAM-1, binds at least in part to carbohydrate structures in the Lewis-x family (12, 13). Lacto-*N*-fucopentaose III appears to be the specific ligand for GMP140 (13). We have shown previously (14) that GMP140 in the fluid phase (which may be similar to the soluble or secreted form) inhibits the binding of activated neutrophils to resting endothelium. Since this adhesion is not mediated by GMP140 but rather the CD18 integrins (15), we hypothesized that the *in vivo* role of soluble GMP140 is to limit the adhesion of circulating neutrophils [polymorphonuclear neutrophils (PMNs)] to endothelium (14).

The possibility that GMP140 has an antiinflammatory effect was investigated by measuring the production of superoxide anions (O_2^-) from PMNs either adherent to immobilized GMP140 or in the presence of fluid-phase GMP140. We show here that immobilized GMP140 and fluid-phase GMP140 inhibit the production of O_2^- generated by PMNs, either unstimulated or when stimulated with the cytokine tumor necrosis factor α (TNF-α) or with the PMN chemoattractant formylmethionylleucylphenylalanine (fMet-Leu-Phe). These results suggest that GMP140 is a protein that inhibits several aspects of neutrophil function associated with inflammation.

MATERIALS AND METHODS

Purification of Human Neutrophils. Neutrophils were purified from normal donors by dextran sedimentation and Ficoll/Hypaque gradient centrifugation as described (16). The cells were resuspended in RPMI 1640 medium (Multisystem, Sydney, Australia) adjusted to pH 7.4 by adding 20 mM Hepes and 2.25% $NaHCO_3$ with antibiotics. Bovine serum albumin (0.1%) (Commonwealth Serum Laboratories, Melbourne, Australia) was added to medium used for superoxide assay and fetal calf serum (2.5%) (Pacific Bioindustries, Sydney, Australia) was used for adhesion assays (adhesion medium). The purity of the PMN preparations were >95% as judged by morphology on cytocentrifuged preparations and >99% viability as judged by trypan blue exclusion.

Stimuli. TNF-α [lot no. S9010AX; specific activity, 6×10^7 units/mg (by TNF-α bioassay)] produced in *Escherichia coli* was supplied by Genentech. A final concentration of 10 units/ml was used in all experiments except where otherwise stated. Endotoxin contamination as judged by the *Limulus* amebocyte lysate assay was <0.72 ng/mg. fMet-Leu-Phe was purchased from Sigma. A stock solution of 1 mM fMet-Leu-Phe in absolute alcohol was stored at −20°C and diluted on the day of each experiment. A final concentration of 0.1 μM was used in all experiments.

Preparation of GMP140. GMP140 was extracted from human platelets and affinity purified; purity was confirmed

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Abbreviations: TNF-α, tumor necrosis factor α; O_2^-, superoxide anion(s); fMet-Leu-Phe, formylmethionylleucylphenylalanine; PMN, polymorphonuclear neutrophil; LEC-CAM, lectin/epidermal growth factor/complement-binding domain cell adhesion molecule.

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by N-terminal sequence analysis as described (17). The purified material was stored in 0.02 M Tris/0.15 M NaCl/0.001 M $CaCl_2$/0.1% Triton X-100 buffer, pH 7.4 (Triton X-100 buffer), and kept at −70°C. For immobilization of GMP140 it was found that removal of Triton X-100 from the preparation was not required provided the wells were washed four times prior to addition of cells. Control wells were coated in Triton X-100 buffer. No change in cell viability was evident with either GMP140- or Triton X-100 buffer-coated wells. For use of GMP140 in the fluid phase, the Triton X-100 was removed by extracti-Gel D (Pierce) on the day of each experiment. The gel was washed in the above buffer without Triton X-100. Two volumes of packed gel to 1 volume of GMP140 was found to be adequate for complete Triton X-100 removal. For controls, the buffer with Triton X-100 removed by extracti-Gel D was used. The concentration of GMP140 was determined by the Bio-Rad (Bio-Rad) protein assay.

Antibodies. Polyclonal antibody to GMP140 was raised in rabbits and Fab fragments were prepared (14). The antibody has been shown to bind specifically to GMP140. Control Fab fragments were made from nonimmune rabbits.

Superoxide Assay. O_2^- release by PMN was measured by the reduction of cytochrome *c* at 550 nm (18). The PMNs were either applied to microtiter wells or incubated in suspensions in Eppendorf tubes. For determination of O_2^- in microtiter wells, flat-bottomed microtiter wells (Nunclon-Delta, Roskilde, Denmark) were either left uncoated or precoated for 2 hr with 30 μl of either fibrinogen (KabiVitrum, Stockholm) or fibronectin (Collaborative Research) at 100 μg/ml or GMP140 at various concentrations. These concentrations of fibronectin and fibrinogen in preliminary assays were found to be saturating. The wells were washed with assay medium four times prior to assay. Human neutrophils (1.5×10^5) in 50 μl were added together with cytochrome *c* (Sigma type VI; final concentration, 110 μM) and stimuli to give a final volume of 100 μl. For determination of O_2^- production in Eppendorf tubes, the same mixture was added to Eppendorf tubes and the cells were incubated in a shaking water bath at 37°C. One hundred microliters of the contents was then transferred to a microtiter plate and the absorbance at 550 nm was determined. For calculation of superoxide dismutase-inhibitable O_2^- generation, a mixture of PMNs, cytochrome *c*, and superoxide dismutase (final concentration, 1 mg/ml) (Boehringer Mannheim) was incubated either in microtiter wells or in Eppendorf tubes. Using the correction factor of Pick and Mizel (19) for estimation of O_2^- production in microtiter wells, the amount of O_2^- produced was determined by reduction of cytochrome *c* measured at 550 nm (using a Dynatech microplate reader). The results are expressed as the amount (nmol) of superoxide dismutase-inhibitable O_2^-.

Hypoxanthine-Xanthine Oxidase System. Hypoxanthine (Sigma) and xanthine oxidase (Sigma) were used to generate O_2^- anion in a cell-free system (20). A 1-ml reaction mixture of 1 mM hypoxanthine and 0.03 unit of xanthine oxidase with or without GMP140 (10 μg/ml) and 620 μM cytochrome *c* was incubated at 37°C and the OD_{550} was determined at 15-min intervals over a 60-min period.

Adhesion Assay. Neutrophils (5×10^5), resuspended in 100 μl of adhesion medium either with or without TNF-α (10 units/ml), were allowed to adhere to flat-bottomed plastic or GMP140-coated microtiter wells for 30 min at 37°C. The medium was then aspirated off and the cells were stained with 0.25% rose bengal for 8 min as described (16, 21). Excess stain and nonadherent cells were removed by washing. The OD was read at 570 nm and the percentage of cells adhering was calculated from a standard curve (21).

Photography of Adherent Neutrophils. Glass slides (Lab-Tek, Nunc) were either uncoated or precoated with 150 μl of GMP140 (10 μg/ml) at 20°C for 2 hr and then washed three times with adhesion medium. Neutrophils (2×10^5) with TNF-α (10 units/ml) in 200 μl of adhesion medium were incubated on the slides at 37°C for 30 min. Nonadherent cells were removed by washing three times in adhesion medium before photographs were taken using an Olympus microscope under 400× magnification. Similar morphology was observed using plastic slides; however glass slides provided better optics for photography.

Endotoxin Assay. A chromogenic *Limulus* amebocyte lysate assay (Coatest, KabiVitrum, Stockholm) with a sensitivity of 0.8 pg/ml was used to measure endotoxin contamination in preparations of fibrinogen, fibronectin, and GMP140.

Statistics. All data were analyzed using unpaired Student's *t* tests.

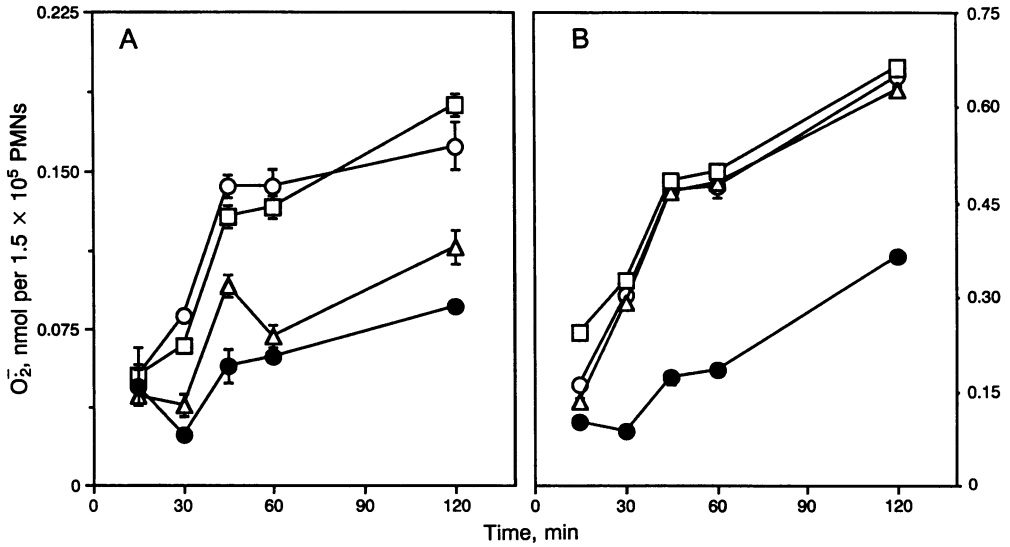

FIG. 1. Time course of O_2^- release on different matrices. Microtiter wells were coated with Triton X-100 buffer (○), fibronectin (100 μg/ml) (△), fibrinogen (100 μg/ml) (□), or GMP140 (10 μg/ml) (●) for 2 hr at room temperature. The wells were then washed four times prior to use. PMNs in medium alone (*A*) or with 10 units of TNF-α per ml (*B*) were then added to the wells and incubated at 37°C. The OD_{550} was read at the indicated times, and the nmol of O_2^- release per 1.5×10^5 PMNs was calculated. Each point represents the arithmetic mean (±SEM) of triplicate determinations from one experiment that is representative of three similar ones performed.

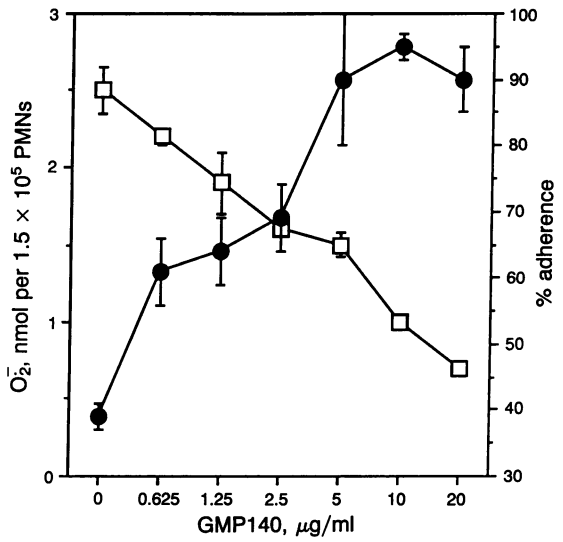

FIG. 2. O_2^- release and percentage of adherent TNF-α-activated PMNs in response to increasing dose of immobilized GMP140. Microtiter wells were coated with increasing concentrations of GMP140 for 2 hr at room temperature followed by four washes in medium. The O_2^- release (□) of TNF-α-activated PMNs was determined after 30 min. In parallel wells the extent of adhesion (●) of TNF-α-activated PMNs was also determined. Each point is the arithmetic mean (±SEM) of triplicate results from one experiment representative of three such experiments.

RESULTS

Effect of Solid-Phase GMP140 on O_2^- Generation by Human Neutrophils. Neutrophils were added to microtiter plates previously coated with GMP140, fibrinogen, or fibronectin in Triton X-100 buffer or control plates of Triton X-100 buffer alone for 2 hr and the plates were washed four times in medium containing 0.1% bovine serum albumin. The O_2^- generation during a 2-hr incubation is shown in Fig. 1. Neutrophils added to wells coated with medium or fibrinogen generated greater amounts of O_2^- than those on GMP140 or fibronectin. No difference in cell viability was observed in PMN adherence to the different matrices. In parallel incubations 10 units of TNF per ml was included. This dose was chosen as it was found to give consistently high levels of adhesion and O_2^- generation of PMNs on plastic (data not shown). As previously described (22), there was a strong O_2^- generation by cytokine-activated PMNs on fibronectin, fibrinogen, and medium. By contrast, O_2^- generation by TNF-α-activated PMNs on GMP140 was absent until 60 min and remained significantly less than on other matrices.

Table 1. Reversal of GMP140 effect by anti-GMP140 antibody

GMP140, μg/ml	Antibody (Fab fragment)	O_2^-, nmol per 1.5 × 10^5 PMNs per 30 min
0	Nil	0.38 ± 0.04
0	Anti-GMP140	0.28 ± 0.02
0	Control	0.32 ± 0.05
3	Nil	0.06 ± 0.03
3	Anti-GMP140	0.34 ± 0.07*
3	Control	0.04 ± 0.01†

Three micrograms of GMP140 was bound to plastic wells for 2 hr at 20°C and excess was removed by four washes with medium. Anti-GMP140 Fab fragment or nonimmune Fab fragment (final concentration of each, 10 μg/ml) was added to the appropriate wells of immobilized GMP140 for 30 min at 20°C; this was followed by washing prior to addition of unstimulated PMNs and cytochrome *c* mix. The amount of O_2^- generated was determined after a 30-min incubation at 37°C. The results show the mean ± SEM of 12 determinations from a pool of four experiments.
*P = 0.02 compared to group receiving no antibody.
†P = 0.004 compared to group receiving anti-GMP140 Fab antibody.

The difference between GMP140 and plastic with unstimulated PMNs at 30 min was observed on 21 occasions with five different preparations of GMP140 (mean inhibition ± SD = 72% ± 16%) and with TNF-α-activated cells on 17 occasions (mean inhibition = 81% ± 13%). At 60 min the differences were 83% ± 4% and 60% ± 20% for unstimulated and TNF-α-stimulated cells, respectively (n = 3 and n = 4). The reduction on GMP140-coated wells in comparison with fibrinogen-coated plates was 74% ± 13% and 74% ± 12%, respectively, at the 30-min time point (n = 7). GMP140 did not alter O_2^- generation in a cell-free system generating O_2^- by hypoxanthine and xanthine oxidase (data not shown).

Relationship of Adhesion to GMP to O_2^- Generation. We have shown previously that unactivated PMNs adhere strongly to immobilized GMP140 (14). TNF-α-activated PMNs also adhere to immobilized GMP140 as is shown in Fig. 2. In parallel wells the O_2^- generation was measured. With increasing amounts of GMP140 immobilized onto microtiter wells there is a dose-dependent increase in adhesion but a dose-dependent decrease in O_2^- generated. Using unactivated PMNs a similar inverse correlation between dose of GMP140 and generation of O_2^- is seen, but the overall level of O_2^- generated is lower than that seen using TNF-α-activated PMNs (data not shown).

The inhibitory effect of GMP140 on O_2^- release could be reversed by rabbit polyclonal anti-GMP140 Fab fragments but not by control Fab fragments, as shown in Table 1. This reversibility also applied to PMN adhesion to GMP140 as shown previously (14).

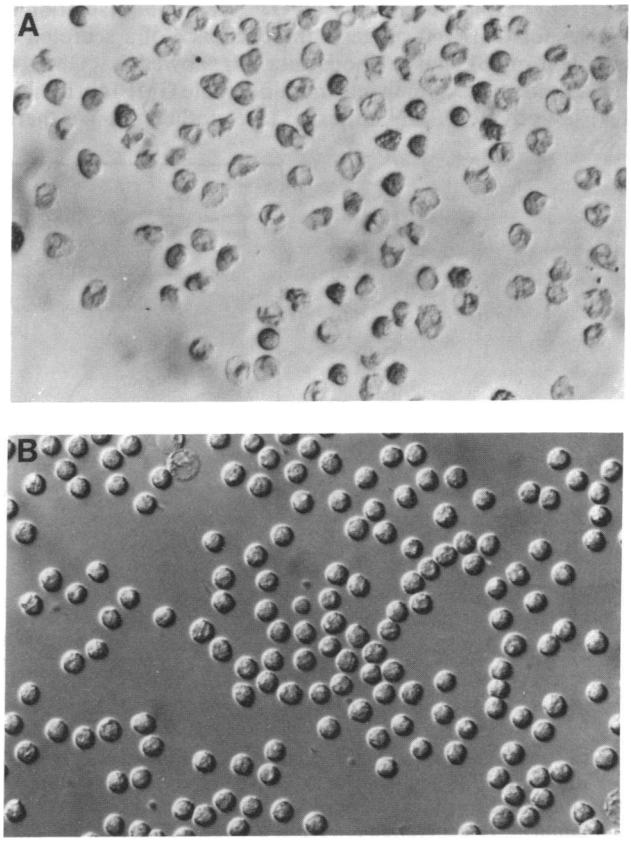

FIG. 3. Morphology of TNF (10 units/ml) activated PMNs on medium-coated glass (*A*) and on GMP140-coated glass (*B*). PMNs (2 × 10^5) were incubated on either medium-coated (*A*) or GMP140-coated (*B*) slides for 30 min at 37°C. Slides were then washed three times to remove nonadherent cells. The cells were maintained under medium at 37°C while photographs were taken. (×456.)

Table 2. Inhibition of O_2^- production by fMet-Leu-Phe-stimulated PMNs adherent to immobilized GMP140

Stimulus	O_2^-, nmol per 1.5 × 10^5 PMNs per 30 min		
	Medium only	Fibrinogen	GMP140
Nil	0.58 ± 0.1	0.86 ± 0.1	0.23 ± 0.07*
fMet-Leu-Phe	1.4 ± 0.1	2.1 ± 0.2	0.64 ± 0.1*
TNF-α	2.1 ± 0.2	1.7 ± 0.1	0.35 ± 0.06*

PMNs with cytochrome *c* were added to microtiter wells coated with medium, 30 μl of GMP140 at 10 μg/ml, or 30 μl of fibrinogen at 100 μg/ml. fMet-Leu-Phe (0.1 μM) or TNF-α (10 units/ml) was then added. O_2^- was measured after 30 min of incubation at 37°C. Results show the mean ± SEM of 12 determinations performed in four independent experiments.

*$P < 0.001$ for unstimulated and fMet-Leu-Phe- or TNF-α-stimulated PMNs applied to GMP140-coated wells compared to groups incubated on medium or fibrinogen-coated plastic.

Microscopic Appearance of PMNs Adherent to GMP140 and Other Matrices. It has been well described that PMNs adhering to plastic or glass become flattened and polarized (23). We found this process could be exaggerated by TNF-α treatment (Fig. 3*A*). By contrast, PMNs adhering to GMP140 remain rounded and refractile and failed to polarize even in response to TNF-α treatment (Fig. 3*B*).

Effect of fMet-Leu-Phe on O_2^- Generation by PMNs Adherent to GMP140. Table 2 shows the results of four experiments in which O_2^- generation by PMNs was measured on plastic, fibrinogen, and GMP140-coated wells in response to 0.1 μM fMet-Leu-Phe (a dose previously determined to give maximal O_2^- generation) or TNF-α. The O_2^- generated in response to both stimuli was reduced on GMP140.

Effect of Fluid-Phase GMP on O_2^- Generation. Isolation of mRNA for GMP140 suggests the existence of a secreted or soluble form (8). Our previous data (14) showed that GMP140 in the fluid phase (which may mimic soluble GMP140) inhibits TNF-α-activated PMN adhesion to endothelium. We therefore tested the effects of fluid-phase GMP140 on generation of O_2^- by PMNs.

Neutrophils were mixed with GMP140 before placement in microtiter wells with or without TNF-α. As shown in Fig. 4, there was a dose-dependent decrease in the capacity of cells incubated with GMP140 to generate O_2^-.

Antibodies to GMP140 inhibited the effects of fluid-phase GMP140 on the O_2^- production by PMNs although the results were inconsistent for reasons not known at present. The most consistent effects for the reversal of the GMP140-mediated inhibition of O_2^- were observed when a suboptimal dose of GMP140 was preincubated with anti-GMP140 Fab antibodies in Eppendorf tubes prior to addition of PMNs, cytochrome *c*, and TNF-α (Fig. 4*B*).

Reversibility of GMP140. To test whether the effect of GMP140 was reversible, PMNs were incubated with GMP140 or with medium for 10 min followed by two washes in medium. Parallel tubes were centrifuged twice and either resuspended in existing medium or washed with fresh medium. The O_2^- generation in response to TNF-α was measured in microtiter plates. As seen in Fig. 5, PMNs in the presence of GMP140 failed to generate significant amounts of O_2^- (group a compared to group b). This inhibition was reversed by washing (group d) but was restored by adding GMP140 back to the cells at the end of the washing process (group e). These effects were evident over 120 min, at which time there was <10% recovery of O_2^- generation by PMNs in the presence of GMP140. The PMNs that were incubated in GMP140 for this 120 min were still viable as judged by trypan blue exclusion (data not shown).

DISCUSSION

The central finding of this paper is that GMP140 interacts with human neutrophils in a way that prevents or does not allow the generation of O_2^-. We have previously shown that GMP140 appears to have a signaling role to PMNs in inhibiting the CD18-dependent adhesion of activated cells to

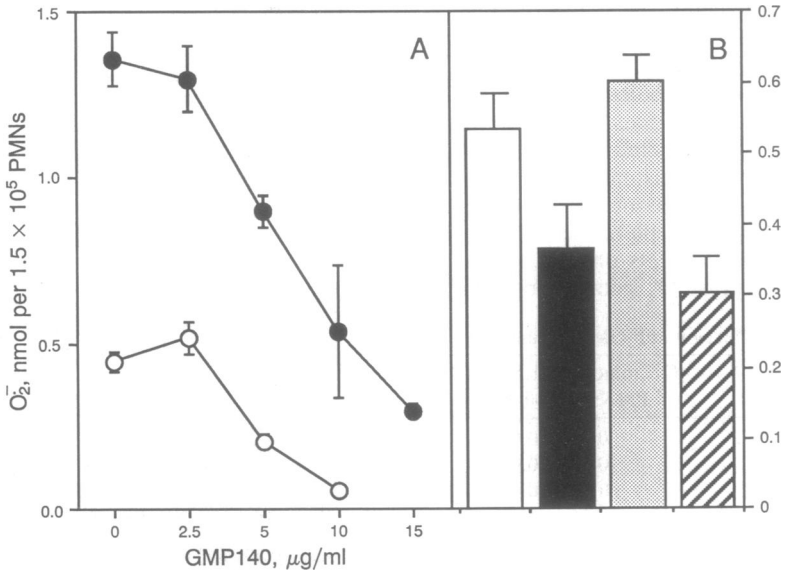

FIG. 4. (*A*) Inhibition of O_2^- production by fluid-phase GMP140. Neutrophils were incubated with control buffer or graded doses of GMP140 for 10 min at 37°C and then added to plastic microtiter plates either with (●) or without (○) TNF-α. O_2^- generation was measured at 30 min. Results show the mean ± SEM of 8–12 determinations pooled from four experiments. (*B*) Anti-GMP140 antibody reverses the GMP140-mediated inhibition of O_2^- generation of TNF-α-activated PMNs. PMNs and cytochrome *c* with TNF-α (5 units/ml) and with or without GMP140 (3 μg/ml) were incubated in Eppendorf tubes in a shaking water bath at 37°C for 30 min. The contents of the tube were then resuspended and transferred to microtiter wells and the OD_{550} was determined. In groups containing anti-GMP140 Fab or control Fab fragments, GMP140 had been incubated with these antibodies for 30 min before addition to the PMNs. Results are the mean ± SEM of six determinations performed in two experiments. Open bar, no GMP140; solid bar, GMP140 (3 μg/ml); stippled bar, GMP140 (3 μg/ml) with anti-GMP140 Fab fragments (40 μg/ml); striped bar, GMP140 (3 μg/ml) with nonimmune Fab fragments (40 μg/ml). $P = 0.035$ for the group incubated with GMP140 compared with no GMP140 (solid vs. open bars); $P = 0.003$ for the group incubated with anti-GMP140 antibody and GMP140 compared with GMP140 alone (stippled vs. solid bar); $P = 0.37$ for the group incubated with control Fab antibody and GMP140 compared with GMP140 alone (striped vs. solid bar).

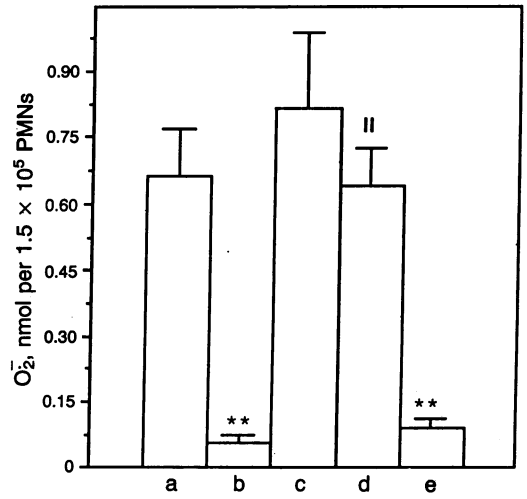

FIG. 5. Inhibition of O_2^- production by GMP140 is reversible. PMNs were incubated with GMP140 (20 μg/ml) (b, d, and e) or buffer (a and c) for 10 min at 37°C. Groups a and b were centrifuged twice but resuspended each time in the existing medium. A solution containing cytochrome *c* and TNF-α (10 units/ml) was then added to the tubes. Groups c–e were washed twice in fresh RPMI medium with bovine serum albumin and then resuspended in the cytochrome *c*/TNF-α mixture. To group e, a further 20 μg of GMP140 per ml was also added. All groups of cells were then transferred to microtiter wells and incubated for 60 min at 37°C, at which time the level of O_2^- production was calculated. The results show the mean ± SEM of nine determinations from three experiments (a–d) and six determinations from two experiments (e). **, $P \leq 0.001$ compared to group a; ||, $P > 0.1$ compared to group c.

endothelium (14). We now extend these findings and show that PMNs that adhere to GMP140 generate little or no O_2^-.

The adhesion of TNF-α-activated neutrophils to GMP140 is qualitatively different from their adhesion to other matrices, such as the extracellular proteins, fibrinogen, and fibronectin, and to plastic surfaces. First, activated PMNs adhering to GMP140 remain rounded and refractile (Fig. 3*B*) rather than spread and polarized as is seen on plastic (Fig. 3*A*) or on fibrinogen and fibronectin (data not shown). Second, adhesion of TNF-α-activated PMNs to GMP140 results in only low levels of O_2^- generation in contrast to their adhesion to fibronectin, fibrinogen, and plastic surfaces, which result in strong O_2^- generation (Fig. 1*B*). The effect of GMP140 on PMN function is best seen in Fig. 2, where increasing amounts of immobilized GMP140 on plastic wells, although leading to an increase in PMN adhesion, result in a decrease in the generation of O_2^-. Interestingly, the morphology of PMNs adhering to GMP140 is similar to that seen for PMNs adhering to endothelium, a situation in which O_2^- generation is also limited (23).

The capacity of GMP140 to inhibit O_2^- generation from activated PMNs (rather than a failure to stimulate it) is seen in two types of experiments. First, PMNs adhering to immobilized GMP140 produce less O_2^- in response to soluble stimuli such as the bacterial chemotactic peptide fMet-Leu-Phe. Second, fluid-phase GMP140 inhibits O_2^- generation by TNF-α-activated PMNs that adhere to plastic (Fig. 4) or other surfaces (not shown). Thus it would appear that GMP140, whether in the fluid phase or bound to plastic, significantly alters the capacity of PMNs to respond to agents that stimulate O_2^-. The PMNs are viable as judged by the vital dye trypan blue exclusion and return to full functional status after the removal of GMP140 by washing (Fig. 5).

Using unactivated PMNs, the effect of GMP140 on O_2^- production is not dissimilar to the situation in which PMNs adhere to fibronectin-coated surfaces where little O_2^- is generated (22). Our findings with fibrinogen-coated plates are somewhat different from published results (22) that show little O_2^- generation on this matrix. Since our preparation of fibrinogen contains 1 ng of endotoxin per ml, this may be responsible for the conflicting results.

Our experiments suggest a new function for GMP140, an adhesion protein of the LEC-CAM family that is only found in the Weibel–Palade bodies of endothelial cells and the α granules of platelets. GMP140 is normally elaborated after activation of endothelium by agents such as thrombin or histamine or by thrombin activation of platelets. GMP140, perhaps in the membrane-bound form, may have a role in maintaining neutrophils in an unactivated state at a time when thrombotic or allergic reactions are taking place. Our observations could also explain the observation that PMNs adhering to endothelial cells *in vitro* generate diminished O_2^- (23) and the lack of damage to the endothelium in thrombotic conditions. In addition, detection of $\approx$0.2–0.3 μg of GMP140 per ml in ultracentrifuged plasma (100,000 × *g*) (M.C.B., L. Dunlop, and M.P.S., unpublished data) suggests that soluble GMP140 may be a normal blood constituent and may tonically prevent release of O_2^- by blood-borne neutrophils until adhesion and migration to sites of inflammation occur. The reversibility of the effects of GMP140 (Fig. 5) would suggest that rapid reversal of the functional state of neutrophils will take place. Our results have implications for prevention of activation of other blood cells, such as eosinophils and basophils, which also adhere to GMP140 (M.A.V., C.M.L., A. Lopez, and J.R.G., unpublished data), and suggest that this natural protein or its derivatives may constitute a powerful therapeutic antiinflammatory agent.

We thank Genentech for the generous gift of TNF-α and Mrs. Mari Walker for preparation of the manuscript. This work was supported by grants from the National Health and Medical Research Council of Australia and the National Heart Foundation. C.S.W. and M.P.S. are recipients of National Health and Medical Research Council Postgraduate Fellowships and M.C.B. is the recipient of a Wellcome Senior Research Fellowship.

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