Mechanisms of Contraction Induced by Human Leukocytes in Normal and Atherosclerotic Arteries

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Activation of leukocytes results in the release of a variety of vasoactive substances that may modulate vascular tone. We studied the effect of human polymorphonuclear (PMN) and mononuclear (MONO) leukocytes on quiescent femoral arteries in vitro. Arteries were obtained from normal and atherosclerotic cynomolgus monkeys. In normal arteries, stimulation of PMNs (3 and 5×10⁶ cells/ml) with either thrombin (5 units/ml) or complement C5a (0.5 µg/ml) resulted in endothelium-independent contraction (approximately 25% of maximum contraction with 80 mM KCl). Vasoconstriction was augmented in the presence of superoxide dismutase (150 units/ml) and was significantly impaired in the presence of the hydroxyl radical scavengers mannitol (20 mM) and deferoxamine (1 mM). Catalase (1,200 units/ml) or l-alanine (20 mM) did not modify this effect of PMNs. In contrast to PMNs, vasoconstriction in response to MONOs was not altered by the addition of radical scavengers. Pretreatment of PMNs and MONOs with indomethacin (10 µM) or nordihydroguaiaretic acid (20 µM) did not influence vascular responses. Supernatant of thrombin-stimulated PMNs and MONOs also produced vasoconstriction (approximately two thirds of the effect of intact cells). This vasoconstrictor factor (or factors) was stable (30 minutes, 95°C) and had a molecular weight < 1,000 as determined by ultrafiltration. Stimulation of MONOs or PMNs (3 and 5×10⁶ cells/ml) produced a similar response in normal arteries. In contrast, the constrictor response in atherosclerotic arteries to MONOs (5×10⁶ cells/ml) was significantly greater than to PMNs. We conclude that stimulated human PMNs and MONOs contract arteries in vitro by release of at least two factors. One factor appears to be heat stable, with a molecular weight < 1,000. The vascular response to PMNs, but not to MONOs, appears to involve the generation of hydroxyl radicals. The response to MONOs is greater than the response to PMNs in atherosclerotic, but not in normal, arteries. (Circulation Research 1991;69:871–880)

Leukocytes, in particular mononuclear cells (MONOs), commonly are found in atherosclerotic arteries.1,2 The interaction of monocyte-macrophages with endothelial and smooth muscle cells appears to play a central role in the development and progression of atherosclerosis.1,2 In addition, stimulation of leukocytes results in the release of several vasoactive substances.3–8 Thus, it is possible that interaction of leukocytes or their products with blood vessels may contribute to modulation of vascular tone by the release of one or more substances.

We have observed that activation of leukocytes in vivo with the chemotactic peptide f-MLP9 or the complement anaphylatoxin C5a10 produces constriction of large arteries in atherosclerotic, but not in normal, primates. The precise mechanism for this striking difference in response to stimulation of leukocytes in atherosclerotic and normal primates is unclear. We have suggested that vascular responses to potential mediators of leukocyte-induced vasoconstriction, such as thromboxane A₂ and prostaglandin E₂, are augmented in atherosclerotic primates.9,11 Although normal and atherosclerotic arteries in vivo respond differently to some vasoactive substances released by leukocytes, studies have not been per-
formed to examine systematically mechanisms by which responsiveness to atherosclerotic arteries is altered.

Previous researchers have examined vascular effects of polymorphonuclear leukocytes (PMNs) on normal arteries in vitro. Depending on the leukocyte preparation technique, experimental protocol, and animal species, different mechanisms for effects of PMNs on vascular tone have been found. Rat peritoneal PMNs or human PMNs appear to relax rat aorta by the release of nitric oxide or a related nitric oxide–containing compound. In contrast, rabbit PMNs appear to contract rabbit aorta either directly, by release of a stable factor, or indirectly, by release of superoxide anions that inactivate endothelium-derived relaxing factor. A different vasoconstrictor mechanism has been proposed for canine PMNs and canine coronary arteries that involves release of lipoygenase products.

In the present study, we examined responses to human leukocytes in femoral arteries in vitro. Novel approaches in this study include the use of arteries from normal and atherosclerotic monkeys and the use of subpopulations of leukocytes (PMNs and MONOs). We studied the vascular effects of subpopulations of leukocytes, because circulating leukocytes are composed predominantly of PMNs, whereas MONOs are the major leukocyte cell type found in atherosclerotic lesions. We studied vascular responses to leukocytes in normal and atherosclerotic arteries, because a difference in the responsiveness may contribute to our previous findings in vivo.

Materials and Methods

Leukocyte Preparation

Heparinized venous blood (300 ml, 20 units/ml) was drawn by antecubital venipuncture (19-gauge needle) from healthy volunteers. Blood was mixed with an equal volume of 3% dextran T500 (diluted in 0.9% NaCl) and incubated for 18 minutes at room temperature to precipitate red cells. The leukocyte-rich supernatant was centrifuged for 10 minutes at 20°C (1,600 rpm, model TJ-6, Beckman Instruments Inc., Fullerton, Calif.). Pellets were resuspended in Hanks' solution, and PMNs and MONOs were separated by a Ficoll-Hypaque density gradient centrifugation (9.97% Hypaque, 6.35% Ficoll, specific gravity 1.08, 1,400 rpm for 40 minutes at 20°C) as described previously.

The enriched PMN and erythrocyte cell fraction was mixed with ice-cold double-distilled water for 20 seconds to lyse remaining red cells. Isotonicity was restored with 1.7% NaCl. Cells were washed twice with Hanks' solution (1,000 rpm for 6 minutes) and resuspended in Krebs' buffer at a final concentration of 10^7 cells/ml. Platelet contamination was 19±0.8×10^3 platelets/µl (n=49). The PMN-enriched fraction consisted of more than 95% PMNs.

The enriched MONO fraction was diluted in Hanks' solution and incubated with human serum containing 5.4 mM EDTA for 10 minutes at 37°C to minimize platelet contamination. Cells were washed twice with Hanks' solution (1,400 rpm, 10 minutes) and resuspended in Krebs' buffer at a final concentration of 10^7 cells/ml. Platelet contamination was 14±0.7×10^3 platelets/µl (n=26). Platelet contamination was significantly less in the MONO than PMN preparation (p<0.001). The MONO fraction consisted of approximately 30% monocytes, 65% lymphocytes, and 5% neutrophils (average count from four cytocentrin preparations).

The MONO and PMN cell suspensions were stored during the experiments at 4°C and were rotated continuously to prevent spontaneous aggregation of leukocytes.

The vascular response to human platelets was tested at a concentration (25,000 platelets/µl) similar to the platelet contamination in our leukocyte fractions. Human platelets were obtained from 100 ml citrated freshly drawn blood from healthy volunteers as described previously. Blood was centrifuged (200g, 10 minutes, 37°C) to obtain platelet-rich plasma, which was washed twice in Hanks' balanced salt solution without calcium. Platelets finally were resuspended in Krebs' buffer and stored under identical conditions as the leukocytes before use.

Leukocyte Viability and Function

Cell viability in each fraction was determined by trypan blue dye exclusion. The viability usually exceeded 95%. Cell fractions with less viability were discarded.

Functional responsiveness was determined by leukocyte aggregation. Aggregation was performed with a dual-beam aggregometer (Payton Scientific Inc., Buffalo, N.Y.) connected to a recorder. Aliquots (0.5 ml) of PMNs or MONOs were added to siliconized cuvettes containing a stir bar revolving at 500 rpm at 37°C. After 5 minutes of incubation, either f-MLP (0.13 µM), C5α (0.5 µg/ml), or thrombin (5 units/ml) was added. The change in light transmission was recorded continuously. Cell fractions that did not show aggregation were excluded.

In four preparations, we measured release of oxygen-derived radicals using electron spin resonance (ESR) spin trapping, as described elsewhere. ESR detection of spin adducts was performed using a Bruker ESP-300 spectrometer. Briefly, aliquots (0.5 ml) of unstimulated or stimulated leukocyte cell suspensions were transferred to a quartz ESR flat cell that was placed in the cavity of the ESR spectrometer. ESR scans were obtained at 25°C. The spin trapping agent was 5,5-dimethyl-1-pyrroline N-oxide at a concentration of 0.1 mM. ESR spectrometer settings were microwave power, 20 mW; modulation frequency, 100 kHz; and modulation amplitude, 1.0 G. Figure 1 shows a spin trap experiment for PMNs and MONOs in the presence and absence of superoxide dismutase and catalase. These spin trap experiments confirmed that PMNs, and to a somewhat
content. The plasma cholesterol and triglyceride contents were, respectively, 129±7 and 22±4 mg/dl in normal primates and 666±43 and 27±5 mg/dl in atherosclerotic animals.

Studies In Vitro

The femoral artery was excised and cut into 4–5-mm ring segments. The vessel preparations were suspended in a siliconized organ bath in 10 ml modified Krebs' buffer (composition in mM: NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, EDTA calcium 0.026, glucose 11.1, and albumin 0.1%, pH 7.40) aerated with 95% O2–5% CO2 and maintained at 37°C. Tension was recorded with a linear force transducer (model FT03c, Grass Instruments, Quincy, Mass.). Over a 1-hour period, the resting force gradually was increased and the artery frequently was exposed to 80 mM KCl until the optimal tension (approximately 2.0 g) for generating force during isometric contraction was reached. The vessels were left at this resting tension throughout the remainder of the study. In some experiments, the endothelium was removed by gently rubbing the endothelial surface with a wooden applicator. Removal of endothelium was confirmed by the absence of dilator responses to acetylcholine (1 μM).

Leukocytes were added to the organ bath in a volume of 3 or 5 ml (final concentration, 3 or 5×106 cells/ml). Leukocytes usually were stimulated with thrombin (5 units/ml final concentration) or, in a few experiments, with complement C5a (0.5 μg/ml final concentration). Both thrombin and C5a stimulate leukocyte chemotaxis and aggregation.23–25 Thrombin and C5a were added to the organ bath 5–15 minutes before addition of leukocytes. This incubation time was chosen because both substances occasionally produced a small vasoconstrictor effect in quiescent vessels. The vasoconstrictor effect was transient, and vascular tone returned to baseline within 10–15 minutes.

It was not possible to study both normal and atherosclerotic arteries on the same day. Because there was substantial variation in responses to activated leukocytes from different donors, we did not perform a direct comparison of leukocyte-induced contraction between normal and atherosclerotic arteries. Because PMNs and MONOs were isolated under identical conditions from each donor, we compared contraction to PMNs with that to MONOs in normal arteries and in atherosclerotic arteries.

In some experiments, leukocytes were preincubated for 15 minutes with 10 μM indomethacin or 20 μM nordihydroguaiaretic acid (NDGA) before use. These concentrations of indomethacin and NDGA have been shown previously to inhibit the release of cyclooxygenase and lipoxygenase metabolites of the arachidonic acid pathway from leukocytes.26–28 In other experiments, vessel preparations were preincubated for 20 minutes with superoxide dismutase (150 units/ml) to scavenge superoxide anions,29 catalase (1,200 units/ml) to scavenge hydrogen peroxide, de-

Animals

Two groups of adult male cynomolgus monkeys were studied. Eighteen normal monkeys were fed commercial laboratory chow (Ralston Purina, Richmond, Ind.). Twelve monkeys were fed an atherogenic diet, which contained cholesterol (1 mg per calorie) and fat (43% of total calories), for 21±2 months (mean±SEM). This feeding protocol has been shown in previous studies to produce typical atherosclerotic lesions (fibrofatty intimal thickening with focal necrosis).21,22 Monkeys weighed 6.1±0.4 kg in the normal group and 6.9±0.3 kg in the atherosclerotic group. At intervals of 3–4 months, monkeys were sedated with ketamine hydrochloride (10 mg/kg i.m.), and a venous blood sample was obtained for measurement of total cholesterol and triglyceride lesser extent MONOs, produce oxygen-derived free radicals under the present experimental conditions.
feroxamine (1 mM) or mannitol (20 mM) to scavenge hydroxyl radicals,\textsuperscript{30} and L-alanine (20 mM) to scavenge hypochloric acid.\textsuperscript{31} Leukocytes were added in the presence of these scavengers. After every intervention, maximal contraction was induced with 80 mM KCl. Repeat exposure to leukocytes did not impair endothelium-dependent relaxation to acetylcholine or contractile responses to KCl.

Supernatant was obtained from leukocytes \((5 \times 10^6 \text{ cells/ml})\) by centrifugation \((1,200 \text{ rpm, 1 minute, } 4^\circ\text{C}).\) Some leukocytes were stimulated with either thrombin \((5 \text{ units/ml})\) or C5a \((0.5 \mu\text{g/ml})\) before centrifugation. In other experiments, supernatant from stimulated or unstimulated leukocytes was passed through an ultrafiltration unit with filters of different molecular weight cutoff \((\text{YM1; Amicon, Beverly, Mass.}).\) To separate effects of thrombin and C5a from responses to supernatant of activated leukocytes, vessels were incubated \((15 \text{ minutes})\) with thrombin before the addition of the thrombin-containing supernatant and with C5a before the addition of C5a-containing supernatant.

**Materials**

Bovine copper-zinc superoxide dismutase, bovine catalase, deferoxamine mesylate, L-alanine, indomethacin, NDGA, mannitol, and human recombinant complement C5a were obtained from Sigma Chemical Co., St. Louis, Mo. Thrombin was obtained from Parke-Davis, Morris Plains, N.J.

**Statistical Analysis**

Data are presented as mean±SEM. Vascular responses to leukocytes are expressed as percent of the maximal response obtained with 80 mM KCl. When the same intervention was performed in several vessels from one animal, responses were averaged so that the number of experiments refers to the number of animals. Student’s \(t\) test for unpaired and paired groups was used. Statistical significance was considered as \(p<0.05.\)

**Results**

**Thrombin- and C5a-Stimulated Leukocytes**

In the presence of thrombin, PMNs and MONOs \((3 \times 5 \times 10^6 \text{ cells/\mu l})\) produced significant contraction of quiescent femoral arteries in vitro (Figures 2, 3, and 4). Vasoconstriction was maximal within 5–10 minutes (Figure 2). Unactivated PMNs and MONOs produced only a small vasoconstrictor effect of approximately 10% of the maximal response obtained with KCl (Figures 3 and 4). The contractile response to PMNs and MONOs was endothelium independent (Figures 3 and 4). C5a-activated PMNs and MONOs \((5 \times 10^6 \text{ cells/ml})\) contracted intact femoral arteries by 27±10% \((n=9; \text{ percent maximum of KCl})\) and 36±14% \((n=6),\) respectively. Thus, stimulation of PMNs and MONOs with C5a produced vasoconstriction similar to that with thrombin.

![Figure 2. Original recordings of response to polymorphonuclear cells (PMN) \((5 \times 10^6 \text{ cells/ml})\) and to mononuclear cells (MONO) \((5 \times 10^6 \text{ cells/ml})\) in normal femoral arteries. Leukocytes were added in the presence of 5 units/ml thrombin. After a contraction plateau was reached, maximal vascular responses were obtained with 80 mM KCl.](http://circres.ahajournals.org/)

**Role of Eicosanoids**

Pretreatment of PMNs and MONOs with indomethacin \((10 \mu\text{M})\) did not alter the vasoconstrictor response to leukocytes in the presence of thrombin. The PMN- and MONO-induced vasoconstrictions were, respectively, 31±7% \((n=11; \text{ percent maximum of KCl})\) and 32±7% \((n=8)\) before indomethacin pretreatment and 37±8% and 30±6% after indomethacin pretreatment. Similar results were obtained with NDGA \((20 \mu\text{M}).\) The PMN- and MONO-induced vasoconstrictions were, respectively, 30±6% \((n=10; \text{ percent maximum of KCl})\) and 22±5% \((n=5)\) before NDGA pretreatment and 29±9% and 33±17% after NDGA pretreatment. These findings suggest that the vasoconstrictor response of leukocytes is unlikely to be mediated by metabolites of the cyclooxygenase and lipooxygenase pathways.

**Oxygen-Derived Free Radicals**

Vasoconstriction in response to stimulated PMNs was enhanced in the presence of superoxide dismutase and significantly impaired in the presence of the hydroxyl radical scavengers mannitol and deferoxamine (Figure 5). The PMN response was not inhibited by catalase, which scavenges hydrogen peroxide, and by L-alanine, a scavenger for hypochloric acid (Figure 5). These findings suggest that vasoconstrictor response to PMNs involves the generation of hydroxyl radicals. Because hydrogen peroxide is an important source for the generation of hydroxyl radicals, we also studied the effect of hydrogen peroxide on monkey femoral arteries. Hydrogen peroxide produced endothelium-independent vasoconstriction (Figure 6). In the presence of deferoxamine \((1 \text{ mM},\)
Normal Versus Atherosclerotic Arteries

Thrombin-activated MONOs and PMNs produced a similar vasocontractor response in normal arteries. In contrast, in atherosclerotic arteries, the vasocontractor response was significantly greater in response to MONOs than to PMNs (Figure 8).

Stable Vasocontractor Factor

Supernatants of unstimulated PMNs and MONOs produced only a small contraction in normal arteries. This constrictor effect was significantly enhanced when the leukocytes were stimulated with thrombin before centrifugation (Figure 9). The supernatant from stimulated PMNs produced approximately 68% and from stimulated MONOs approximately 78% of the vasoconstrictor response that could be obtained with stimulated cells (Figures 9 and 10). The supernatant could be stored at −28°C for 2 days without loss of vasoactivity. The vasoconstrictor response of the supernatant obtained from PMNs (n=2) and MONOs (n=6) was not impaired by heating for 30 minutes at 95°C. Contraction was preserved when the supernatant of stimulated PMNs and MONOs was passed through filters with a molecular weight cutoff of 1,000. These findings suggest that both PMNs and MONOs release a heat-stable factor or factors with a molecular weight <1,000 that accounts for more than two thirds of the vasoconstriction obtained with stimulation of the intact cells.

Contaminating Platelets

Leukocytes were diluted 1:1 by adding to the organ bath, and the final concentration of contaminating platelets was <10,000 platelets/μl. Washed human
platelets at a concentration of 25,000 platelets/μl produced no response of quiescent normal arteries in the absence or presence of thrombin (n=3).

Discussion

The present study shows first, that both subpopulations of human leukocytes, PMNs and MONOs, produce endothelium-independent vasoconstriction in vitro. Second, the vascular response to MONOs, but not to PMNs, was augmented in atherosclerotic arteries. Third, the vascular response to PMNs, but not to MONOs, may involve the generation of hydroxyl radicals. Fourth, the vascular response to both leukocyte subpopulations does not appear to be mediated by the release of eicosanoids. Finally, both leukocyte subpopulations appear to release a heat-stable vasoconstrictor factor or factors with an apparent molecular weight <1,000.

Contaminating Platelets

Preparation of leukocyte subpopulations by Ficoll-Hypaque density gradient centrifugation reduces but does not completely prevent platelet contamination. Platelets release several vasoactive substances, primarily ADP, serotonin, and thromboxane A2. Thus, contaminating platelets may contribute to the vasoconstrictor response to activation of leukocytes. The possibility that activated platelets produce directly the vasoconstrictor response to activation of leukocytes seems unlikely. First, platelets at the concentration that contaminated the leukocyte preparation did not produce vasoconstriction. Second, human platelets at a higher concentration (100,000 platelets/μl) produce dilatation in arteries with intact endothelium in vitro and only minimal, if any, constriction in denuded human arteries. It appears that the major vasoactive compound of human platelets is ADP and not the vasoconstrictor agonists serotonin and thromboxane.

Third, although contamination with platelets was greater in PMNs than MONOs, the contractile response to PMNs was less than that to MONOs. The present study does not exclude the possibility, however, that contaminating platelets may contribute indirectly to the vasoconstrictor response by a synergistic effect with leukocyte-derived products.

Monocytes Versus Lymphocytes

Addition of leukocytes to the organ bath in the absence of activators produced only a small vascular effect in the present study. When leukocytes were activated by thrombin or complement C5a, PMNs and MONOs contracted normal arteries by approximately 24% and 32% of the maximal effect obtained with KCl, respectively. The MONO fraction consists of both lymphocytes and monocytes. The present study does not allow us to determine whether the contractile response was mediated by monocytes or lymphocytes.

Leukocyte Responses Are Endothelium Independent

The vasoconstrictor response to PMNs and MONOs in the present study was endothelium independent, a finding in contrast to previous reports. Nishida et al demonstrated that vasoconstriction in response to canine PMNs is abolished by endothelial denudation. This effect was greatly suppressed when

**Figure 5.** Effect of radical scavengers on constriction of normal femoral arteries induced by polymorphonuclear cells (PMN). Vessel preparations were pretreated for 20 minutes with either superoxide dismutase (SOD, 150 units/ml), catalase (CAT, 1,200 units/ml), mannitol (MAN, 20 mM), deferoxamine (DEF, 1 mM), or l-alanine (ALA, 20 mM). PMNs (5×10⁶ cells/ml) were added in the presence of 5 units/ml thrombin. Each vessel preparation served as its own control. Constriction is expressed as percent maximal response obtained with 80 mM KCl. Values are mean±SEM. Number of animals is given at the bottom of the bars; *p<0.05 vs. control.

**Figure 6.** Effect of hydrogen peroxide on normal femoral arteries with and without intact endothelium. Constriction is expressed as percent maximal response obtained with 80 mM KCl. Hydrogen peroxide was added cumulatively. Values are mean±SEM of n=6–7 experiments. Maximal response to KCl in intact and denuded vessels was 8.7±2.6 and 7.3±0.5 g, respectively.
the cells, but not the canine vessel preparation, were pretreated with 5-lipoxygenase inhibitors of arachidone. The authors suggested that PMNs release leukotriene A₄, which is transformed in endothelial cells to the vasoactive leukotriene C₄. This mechanism seems to be of minor importance in our model. Pretreatment of PMNs and MONOs with the lipoxygenase inhibitor NDGA did not affect the vascular response in the present study. Furthermore, we have shown in previous studies that large arteries in the limb of monkeys do not constrict in response to leukotrienes.⁹

Ohlstein and Nichols¹⁵ reported that rabbit peritoneal PMNs produce contraction of the rabbit aorta in vitro, which is abolished by endothelial denudation. They suggested that PMNs release superoxide anions that inactivate basally released endothelium-derived relaxing factor, thereby producing contraction. Although the leukocyte subpopulations in the present study also release superoxide anions, as detected by spin trap experiments, the vasoconstrictor effect of PMNs or MONOs in intact arteries could not be blocked by superoxide dismutase. Thus, inactivation of endothelium-derived relaxing factor by leukocyte-derived superoxide radicals does not appear to be important in our model. In fact, the vasoconstrictor response to PMNs was significantly augmented in the presence of superoxide dismutase.

**Mediators of Leukocyte-Induced Vasoconstriction**

The mechanism for PMN- and MONO-induced vasoconstriction in the present study is not clear. It appears that at least two factors are involved in the vascular response. The main vasoconstrictor activity was found in the supernatant of stimulated PMNs or MONOs. The vasoconstrictor factors or factor was heat stable, with an apparent molecular weight <1,000. A recent report by Sessa and Mullane¹⁴ also demonstrated a stable constrictor factor derived from rabbit leukocytes that is endothelium independent, not mediated by superoxide anion or hydrogen peroxide, and apparently is not a cyclooxygenase or lipoxygenase metabolite. The factor obtained from rabbit PMNs has a molecular weight of less than 4,000 Da and accounted for 24–48% of the vasoconstriction produced by activated PMNs. The vasoconstrictor factor or factors released by human PMNs or MONOs in the present study accounted for more than two thirds of the response obtained with the cells. It is not known whether the constrictor factors obtained from rabbit PMNs and from human PMNs and MONOs are identical.

The PMN-induced vasoconstriction in the present study was enhanced in the presence of superoxide dismutase and markedly impaired in the presence of the hydroxyl radical scavengers mannitol and deferoxamine. The concentration of deferoxamine was chosen based on a study³² that showed that high concentrations (>0.5 mM) of deferoxamine were required to protect endothelial cells against neutrophil-mediated killing. These findings suggest that the generation of hydroxyl radicals is involved in the PMN-induced vasoconstriction. Because of their ex-
hydroxyl radicals. This speculation could explain our finding that superoxide dismutase enhanced the contractile effect of PMNs by increasing the concentration of hydrogen peroxide. In fact, hydrogen peroxide contracts monkey arteries. A surprising finding was that catalase, a scavenger for hydrogen peroxide, did not attenuate PMN-induced vasoconstriction. A possible explanation for this finding is suggested by a recent report by Rice and Weiss, who demonstrated that PMNs release their substances directly within the site of contact of leukocytes and endothelial cells. This protected "microenvironment" may not be accessible to large molecules such as catalase.

It is interesting to note that, in contrast to PMNs, the vasoconstrictor response to MONOs does not appear to involve oxygen-derived free radicals. The reason why oxygen-derived free radicals contribute to the vasoconstrictor response to PMNs but not to MONOs is unknown. We did not attempt to quantify release of oxygen-derived free radicals by PMNs and MONOs, but the spin trap experiments suggested that release of oxygen-derived free radicals may be less from MONOs than from PMNs (Figure 1). Thus, smaller amounts of oxygen-derived free radicals released by MONOs may contribute to these differences.

A recent study by Marceau et al. demonstrated that the chemotactic peptides f-MLP and complement C5a produce contraction of human umbilical arteries in vitro. The authors suggested that the contractile response to chemotactic peptides was mediated by macrophagelike cells in the vessel wall. In contrast to our findings, contraction could be inhibited by indomethacin or the thromboxane A2/prostaglandin H2 antagonist SQ-29,548, which suggests that the response was mediated by cyclooxygenase products. We speculate that there may be major differences in mediators of responses to activated nonresident leukocytes (as in the present study) or tissue-resident leukocytes, as in the study by Marceau et al.

Effect of Leukocytes on Atherosclerotic Arteries

Compared with the PMN-induced response, MONOs produced slightly more contraction in atherosclerotic than in normal arteries. This finding is surprising in some ways because, if smooth muscle...
cells in atherosclerotic arteries are exposed chronically to leukocyte-derived factors, one might expect that the vessels would be desensitized instead of being supersensitive. Thus, we speculate that leukocytes in atherosclerotic arteries may not produce contractile factors continuously and may not desensitize the vessel.

The mechanism for the augmented response to MONOs in atherosclerotic arteries is not known, but the implications are of interest. Previous studies showed that activation of leukocytes produces pronounced constriction of large arteries in atherosclerotic, but not in normal, primates in vivo. The present finding that MONOs produce more contraction in atherosclerotic than in normal arteries may contribute to, but not fully explain, the findings in vivo.

In normal animals in vivo, leukocytes are not present in the adventitia and are not adherent to the vessel wall. In the presence of blood flow in vivo, leukocyte-derived vasoactive substances may have little access to the arterial wall, resulting in minor vascular response. In contrast, the presence of leukocytes that are adherent to endothelium or present in the wall of atherosclerotic arteries may facilitate the direct action of leukocytes and their products on the vessel wall in vivo. In the present study in vitro, leukocytes and their products have direct access to smooth muscle and adventitia in both normal and atherosclerotic vessel preparations. Thus, we speculate that at least two factors contribute to the enhanced response to activated leukocytes in atherosclerotic primates in vivo. First, adherence of leukocytes to the endothelium or their presence in the vessel wall may provide a strategically placed source of vasoactive substances, and second, responses of the atherosclerotic vessel wall to MONOs or MONO-derived products are augmented.

Implications

Activation of the complement cascade has been demonstrated previously in arteries obtained from patients with atherosclerosis. Activated complement may be an endogenous activator of either circulating leukocytes or leukocytes localized in the vessel wall. Our present study suggests that stimulated human leukocytes may increase vascular tone by the release of constrictor factors and therefore may play an important role in the pathogenesis of vasospasm.

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References


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