# Oxygen Radicals Mediate Endothelial Cell Damage by Complement-Stimulated Granulocytes

## AN IN VITRO MODEL OF IMMUNE VASCULAR DAMAGE

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ABSTRACT During hemodialysis, alternative pathway complement activation leads to pulmonary sequestration of granulocytes, with loss of pulmonary vascular endothelial integrity and, at times, protein-rich pulmonary edema. An in vitro model of this phenomenon was constructed utilizing $^{51}$Cr-labeled human umbilical vein endothelial cell cultures. In this system, granulocytes, when exposed to activated complement (C), induce endothelial damage; this injury is mediated primarily by oxygen radicals produced by the granulocytes. C5a appears to be the C component responsible for granulocyte-induced cytotoxicity; studies with cytochalasin B-treated granulocytes suggest that close approximation of the granulocytes and endothelial cells is necessary for maximal cell injury.

## INTRODUCTION

Recent studies from our laboratory have demonstrated that alternative pathway activation of the complement (C)[1] cascade occurs in patients undergoing hemodialysis with cellophane membrane equipment (1–3). Associated with such activation, we reported that hypoxemia, hypocapnia, and impaired carbon monoxide diffusion develop in virtually all hemodialyzed patients (3), and that this pulmonary dysfunction results from C-mediated plugging of the pulmonary vasculature with aggregating granulocytes (1, 2). In parallel studies devised to more rigorously examine the pulmonary effects of C activation, sheep were hemodialyzed by reinfusing autologous plasma which had either been passed over dialyzer cellophane or which had been more traditionally C-activated by exposure to zymosan. In either case, striking pulmonary vessel leukostasis and pulmonary arterial hypertension occurred. More intriguingly, pulmonary edema was histologically obvious, and the pulmonary lymph collected from these animals was not the expected simple transudate which is formed when pulmonary venous drainage is purposefully occluded, but was inappropriately rich in protein (3). This suggested that pulmonary endothelial damage might occur during C activation because under normal circumstances, the vascular endothelial lining presents an efficient barrier to significant protein leak. These observations led us to hypothesize that in hemodialyzed patients, pulmonary endothelial tissue might be damaged by a C-directed, granulocyte-mediated inflammatory reaction, and that such damage, when repeated frequently might produce the pulmonary fibrosis/calcinosis syndrome seen in some chronically hemodialyzed patients (4). The studies to be presented document that in an in vitro system of cultured human endothelial cells, granulocytes, if exposed to activated C, can induce endothelial damage; furthermore, this damage is shown to be mainly mediated by toxic oxygen metabolites released by the stimulated granulocytes. In addition, these studies, which have been published in preliminary form elsewhere (5), reveal that C5a is a critical effector of this injury, and that a close physical proximity between granulocytes and endothelium is necessary for the damage to occur. Although provoked by a relatively narrow interest in the pulmonary damage of hemodialysis, we believe our results can be more widely interpreted to suggest mechanisms of vascular damage in various immunologically provoked vasculitities, shock lung, and, perhaps even more generally, may be germane to the understanding of atherosclerosis.

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[1] *Abbreviations used in this paper*: C, complement; PMN, polymorphonuclear leukocyte.

## METHODS

*Preparation of endothelial cells.* Endothelial cells were obtained from human umbilical cord veins by described methods (6). Briefly, the cord was severed from the placenta soon after birth, placed in a sterile container filled with "cord buffer" (0.14 M NaCl 0.004 M KCl, 0.001 M phosphate buffer of pH 7.4, 0.011 M glucose), and stored at 4°C for no longer than 8 h until further processing. The umbilical vein was cannulated with a syringe adapter (Arthur Thomas Co., Philadelphia, Pa.) and polyethylene tubing, and then freed of blood by gentle perfusion with 80 ml of cord buffer. Thereafter, the vein was infused with 10 ml of cord buffer containing 0.2% collagenase (Worthington Biochemical Corp., Freehold, N. J.), clamped shut, and incubated at room temperature for 20 min. The collagenase solution containing the endothelial cells was flushed from the cord with 20 ml of cord buffer; the cells were sedimented and washed once in cord buffer by sequential centrifugations at 250 *g*, then resuspended in 7.5 ml of culture medium[2] and aliquots transferred to three plastic 35-mm petri dishes (3001, Falcon Plastics, Division of BioQuest, Oxnard, Calif.). The dishes were incubated at 37°C under 5% $CO_2$ with twice weekly changes of culture medium. Cultured cells were identified as endothelium by reaction with fluoresceinated rabbit antisera to human Factor VIII antigen, as described (7).

*Preparation of leukocyte suspensions.* Leukocyte suspensions which contained ≅ 98% polymorphonuclear leukocytes (PMN) (free of erythrocytes and platelets) were prepared from heparinized (10 U/ml) venous blood obtained from healthy adults, ABO-matched with endothelial cell donors, by employing standard techniques of dextran sedimentation, hypotonic lysis of erythrocytes, and Ficoll-Hypaque gradient centrifugation (8, 9). The enriched granulocytes were suspended in a Hanks' balanced salt solution (Microbiological Associates, Walkersville, Md.), containing 0.5% human albumin, to a concentration of $1 \times 10^7$ cells/ml and, when appropriate, were preincubated with cytochalasin B (5.0 μg/ml) (ICI Research Laboratories, Alderly Park, Chesire, England) in 0.1% dimethyl sulfoxide, or in dimethyl sulfoxide alone, at 37°C for 10 min before further addition of appropriate other reactants.

*Immune reactants.* ABO-matched serum complement was activated with 2 mg/ml zymosan (Nutritional Biochemicals Corp., Cleveland, Ohio) for 30 min at 37°C. Thereafter, zymosan particles were removed by centrifugation at 3,000 *g* for 10 min, and the resulting activated serum complement used within 1 h of preparation. When required, opsonized zymosan particles were harvested and resuspended in buffered Hanks'/albumin at a concentration of 5.0 mg/ml.

C5a-rich fractions from zymosan-treated plasma were generated by chromatography over Sephadex G-75 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) employing phosphate-buffered saline containing 0.01% sodium azide as the eluent as described (2). The eluted fractions were monitored by measuring (PMN) chemotactic and aggregating activities as described in other studies from our laboratory (2, 10). The active fractions corresponding to an approximate mol wt = 17,000 daltons were pooled as C5a and concentrated to a final volume of 20 ml by ultrafiltration through an Amicon PM-10 filter (Amicon Corp. Scientific Sys Div., Lexington, Mass.). Before use, its identity as C5a was confirmed by showing that its chemotactic and aggregating activities were specifically inhibited by anti-C5 antibody, but not by anti-C3 antibody (see below). In addition, chemotactically active fractions corresponding to an approximate mol wt = 9,000 daltons were pooled and concentrated in a similar fashion. Incubation with anti-C3 antibody abolished the chemotactic activity associated with these fractions.

When appropriate in selected experiments described below, the C5a-rich fractions were inactivated for 30 min at 37°C with an equivalent volume of anti-C5 antibody (lot 2980K, Behring Diagnostics, Somerville, N. J.) as described (2). The resulting immune complexes were removed by centrifugation at 20,000 *g* and the supernatant fraction was used in endothelial cell incubations.

*Determination of endothelial damage.* $^{51}$Cr-labeled endothelial cells were prepared by adding 5 μCi of radiolabeled sodium chromate (≅ 50 mCi/mg, E. R. Squibb & Sons, Inc., Princeton, N. J.) to the cultured cells 16 h in advance of planned experiments. Approximately 20% of the added label was retained after six washes with buffered Hanks' solution. An additional 5–8% of cell-bound $^{51}$Cr spontaneously eluted during the usual 90-min incubations utilized in the described experiments, whereas three freeze-thaw cycles released 99% of the label into the supernatant fluid.

Culture plates containing $1 \times 10^6$ labeled, nearly confluent cells in 35-mm petri dishes were used in endothelial damage experiments within 3–5 days of primary explanting. Controls for spontaneous $^{51}$Cr release consisted of labeled cell cultures which were derived from the same umbilical cord and which were treated identically before addition of specific reactants. These reactants included, where appropriate: (*a*) $1 \times 10^7$ PMN; (*b*) 0.4 ml of zymosan-activated serum complement; (*c*) 0.1 ml of chromatographically enriched and ultrafiltered C5a or "C3a"; (*d*) Hanks'/albumin containing 0.1 mM of xanthine and 0.1 U of xanthine oxidase (Sigma Chemical Co., St. Louis, Mo.); and (*e*) 10 μg/ml of superoxide dismutase (Truett Laboratories, Dallas, Tex.) plus 10 μg/ml of catalase (Sigma Chemical Co.). After making final reaction mixtures to 2.0 ml with Hanks'/albumin, the plates were incubated for 90 min at 37°C.

After incubation, the attached endothelial cells were washed three times with buffered saline and the washes were pooled. The cells were then removed from the culture dishes and the $^{51}$Cr content of the respective fractions were assayed in a gamma scintillation spectrometer. Care was taken to sediment, and recombine with the endothelial fractions, any endothelial cells removed during the washing procedure. The $^{51}$Cr released into the supernate was expressed as a percentage of that released by three freeze-thaw cycles, a procedure which was independently verified to release 99% of bound $^{51}$Cr from the cultured cells.

In ancillary experiments, endothelial cell damage was measured with an unrelated cytotoxicity assay. This technique takes advantage of the fact that fluroescein diacetate (Paultz and Braun, Stanford, Conn.), a nonfluorescent fatty acid ester, passes readily into living cells where it is hydrolyzed to free fluorescein. The latter is both polar and fluorescent, and accumulation of fluorescein by viable cells is termed fluorochromasia. Dead cells remain nonfluorescent (11, 12).

Cytotoxicity was measured as described above, except endothelial cells were not incubated with $^{51}$Cr. At the termination of the experiment, endothelial monolayers were washed free of neutrophils and collected by mild trypsinization (<5% of the residual cells were neutrophils). These endothelial cells were then suspended in Hanks' albumin buffer containing 10 μg/ml of fluorescein diacetate and incubated in the dark at room temperature for 10 min. Fluorochromasia was quantified in an Ortho model 4801 flow microfluorometer (Ortho Instruments, Westwood, Mass.) in which the number of fluorescent

[2] Media 199, containing 20% fetal calf serum, penicillin (200 U/ml), streptomycin (200 mg/ml), and L-glutamine (2 mM), available from Grand Island Biological Co., Grand Island, N. Y.

cells detected is reported as a percentage of total cells. In many experiments utilizing both endothelial cells and L-1210 tumor cells there has been good agreement between this automated technique and visual inspection.

## RESULTS

*Complement-activated PMN damage cultured endothelial cells.* When labeled endothelial cell monolayers are incubated with the combination of PMN and activated serum complement, significant release of $^{51}Cr$ over that of simultaneously incubated control cells is observed; by contrast, the deletion of either PMN or the activated C prevents endothelial damage (Table I). To elucidate the activated C component(s) responsible for triggering PMN-mediated endothelial damage, zymosan-activated plasma was fractionated chromatographically. Fractions enriched with C5a, when incubated with PMN, induce significant $^{51}Cr$ release from cultured endothelial cells; moreover the resulting damage closely reproduces that engendered by an equivalent amount of unfractionated zymosan-activated whole serum, and could be prevented by pretreatment of the C5a fraction with anti-C5 antibody (Table II). In contrast, other chemotactically active chromatographic fractions (mol wt $\simeq$ 9,000, activity blocked by anti-C3 antibody) do not induce significant $^{51}Cr$ release (Table II).

Because of the somewhat small amounts of $^{51}Cr$ which are released in these studies, endothelial damage was assayed by another, unrelated technique—that of fluorochromasia (11, 12). With this technique, C5a or C plus PMN produce a 20–30% loss in endothelial cell viability during the usual 90-min incubation time utilized in these studies (Table III).

*Mechanism of endothelial injury.* To investigate the obvious possibility that endothelial cell damage

TABLE I
*Complement-Activated PMN Damage Endothelial Cells*

| Additions to endothelial cells | (*n*) | $^{51}Cr$ Release* | *P* |
|---|---|---|---|
| | | *% of control* | |
| Activated C + PMN‡ | (9) | 190.9±29.1 | <0.01 |
| Heat-inactivated C + PMN§ | (7) | 120.1±9.3 | NS |
| Untreated Sera + PMN | (4) | 115.0±9.8 | NS |
| Activated C alone | (4) | 104±4.0 | NS |

Significance was determined by the Student's unpaired *t* test.
* Results are expressed as mean percent release of $^{51}Cr$ above control±SEM. Each experiment utilized as the internal control the spontaneous $^{51}Cr$ release from cells harvested from the same umbilical cord. Mean spontaneous release was 5.2%.
‡ Zymosan-activated serum complement.
§ Zymosan-treated serum previously heat decomplemented (56°C for 30 min).

TABLE II
*C5a-Treated PMN Damage Endothelial Cells*

| Additions to endothelial cells* | (*n*) | $^{51}Cr$ Release | *P* |
|---|---|---|---|
| | | *% of control* | |
| C5a + PMN | (6) | 188.5±17 | <0.0025 |
| C5a + anti-C5 + PMN | (4) | 120.3±5.5 | NS |
| C3a‡ + PMN | (4) | 127.0±10.9 | NS |

* C5a was enriched, purified, and inhibited as described in Methods. Results are presented as described in Table I.
‡ C3a refers to low molecular weight chemotactic activity isolated by chromatography of zymosan-treated plasma. The chemotactic activity was inhibited by incubation with anti-C3 antibody (see Methods).

might result from lysosomal enzymes released from C-triggered PMN, we assayed supernatant myeloperoxidase (previously shown not to be present in endothelial cells) as a convenient marker for lysosomal enzyme release. Indeed, when opsonized zymosan particles are presented to PMN atop endothelial cells, large amounts of myeloperoxidase are released by the phagocytosing PMN, and associated endothelial damage is apparent (top line, Table IV). However, such release is not required for endothelial damage to occur; that is, negligible amounts of released myeloperoxidase accompany the significant endothelial damage provoked by PMN stimulated by activated whole serum complement or enriched C5a (middle lines, Table IV). In studies not shown, release of a second lysosomal enzyme, lysozyme, was also found not to correlate with endothelial cytotoxicity.

Upon exposure to immune stimuli, including C5a, granulocytes generate hydrogen peroxide and various toxic oxygen products, including superoxide anions, hydroxyl radicals, and singlet oxygen (13–21), we investigated the possible deleterious effects of these products on endothelial cell cultures. When a model, free radical-generating system of xanthine and xanthine

TABLE III
*Fluorochromatic Confirmation of Endothelial Injury*

| Additions to endothelial cells | (*n*) | Fluorescent cells* |
|---|---|---|
| | | % |
| Untreated sera + PMN | (6) | 93–97 |
| Activated C + PMN | (4) | 73–81 |
| C5a + PMN | (2) | 67–68 |

* As described in references 11 and 12, only viable cells fluoresce when treated with fluoroscein diacetate. Fluorescence was quantitated in an Ortho 4801 flow microfluorometer. The experimental conditions were the same as described in Table I, except $^{51}Cr$ labeling was omitted.

TABLE IV
*Complement-Mediated Endothelial Damage is Independent of Lysosomal Enzyme Release**

| Additions to endothelial cells and PMN | (n) | Endothelial $^{51}Cr$ release | Myeloperoxidase release |
|---|---|---|---|
| | | *% of control* | *%* |
| C-opsonized particles | (4) | 156±10 | 39±1.8 (SE) |
| C-activated sera | (4) | 191±29 | 1.6±1.0 |
| Purified C5a | (4) | 188±17 | 2±0.6 |
| Untreated sera | (4) | 115±10 | 0.6±0.2 |

* The experimental conditions and method of presentation are the same as described in Table I. The depicted percent myeloperoxidase release is compared to the amount released from an equal number of sonicated PMN used in the same experiment.

oxidase is incubated with labeled endothelial cells, striking $^{51}Cr$ release occurs; the addition of only xanthine or xanthine oxidase did not cause $^{51}Cr$ release. Simultaneous addition of superoxide dismutase plus catalase to the complete system, however, reduced $^{51}Cr$ leak to control levels (Fig. 1A). Addition of catalase alone was also protective, whereas superoxide dismutase alone provided only variable and inconsistent protection (not shown). Analogous observations were made with C5a/PMN-provoked endothelial damage, albeit a lesser degree of damage was noted in this case.

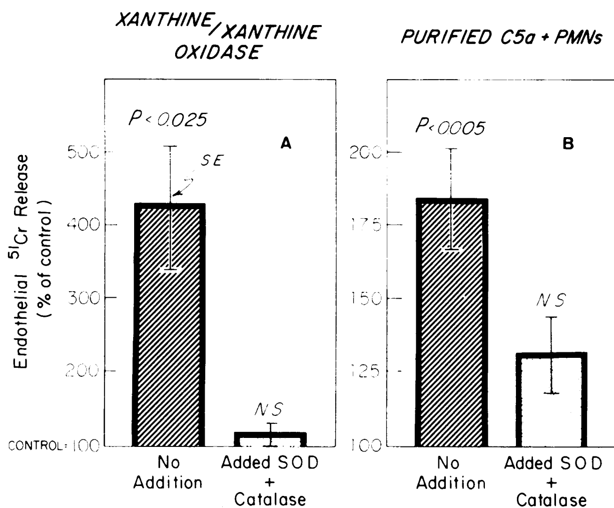


FIGURE 1 Protection of endothelial cell monolayers by superoxide dismutase (SOD) and catalase. (A) $^{51}Cr$-labeled endothelial cells were exposed for 30 min at room temperature to a mixture containing 0.1 mM xanthine and 0.1 U xanthine oxidase with (stippled bar) or without (hatched bar) added superoxide dismutase (10 $\mu$g) and catalase (10 $\mu$g). (B) $^{51}Cr$-labeled endothelial cells treated with PMN ($1 \times 10^7$) and C5a-enriched fractions for 90 min at 37°C with (stippled bar) or without (hatched bar) superoxide dismutase and catalase. Results expressed as in Table I.

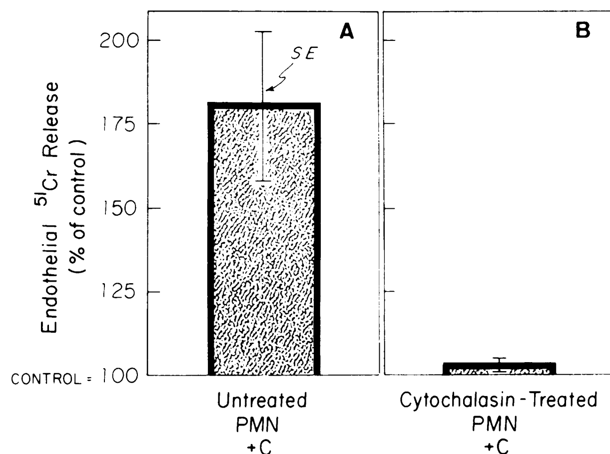


FIGURE 2 $^{51}Cr$ release from endothelial cells exposed to untreated or cytochalasin B-treated PMN. (A) Endothelial cells overlayed with $1 \times 10^7$ PMN plus 0.4 ml zymosan-activated serum complement (C). (B) endothelial cells exposed as above, PMN pretreated with cytochalasin B (5.0 $\mu$g/ml) for 30 min as in Methods. All cell mixtures were incubated for 90 min at 37°C. Results are expressed as in Table I.

As in the xanthine oxidase model system, C5a-triggered, PMN-mediated endothelial damage is markedly inhibited by addition of superoxide dismutase plus catalase (Fig. 1B). Similar results were obtained utilizing activated whole serum C as well (not shown). The greater cytotoxicity produced by the xanthine oxidase system reflects at least a threefold greater production of superoxide than that generated by C-triggered granulocytes (data not shown).

The fact that superoxide (as measured by cytochrome *c* reduction [17]) and other free radicals are generally short-lived and rapidly dissipated, suggests that a close apposition of activated granulocytes to target endothelial cells should be necessary for significant cellular damage to occur, if indeed these toxic oxygen products are critical to such damage. To test this hypothesis, granulocytes were pretreated with cytochalasin B before placing them on endothelial cells in the presence of activated serum complement. This fungal metabolite prevents complement-induced granulocyte spreading (22–25) and, in studies not shown, strikingly decreases adherence of C-treated PMN to both endothelial cells and plastic petri dishes.[3] Associated with cytochalasin treatment and the presumed resulting diminution in

[3] In ancillary studies, cytochalasin treatment decreased by 42% the adherence of C-activated PMN to plastic petri dishes. More relevant to the present studies, cytochalasin-treated neutrophils were by microscopic evaluation also far less adherent to endothelial monolayers. That is, after a single wash, one-half as many cytochalasin-treated granulocytes adhered to endothelium as did similarly washed untreated C-stimulated granulocytes.

effector-target cell interaction, endothelial damage by C-triggered PMN is prevented (Fig. 2).

## DISCUSSION

Several systemic disease states, exemplified by systemic lupus erythematosis, appear to result from the generalized activation of the mediators of inflammation by focal insults. A more recently described, and previously unsuspected, iatrogenic example of this phenomenon is noted in patients undergoing hemodialysis with cellophane membrane equipment—a procedure which has been shown to activate the complement cascade by the alternative pathway (1–3). Accompanying such complement activation, granulocyte sequestration in pulmonary vessels occurs which eventuates in pulmonary dysfunction marked by the appearance of an exudative interstitial edema (3). In this situation, and others characterized by ongoing complement activation, we have hypothesized, and provided supporting data (2), that complement-activated PMN adhere to blood vessel endothelial lining. In the present studies, we have addressed the further question as to whether such complement-activated PMN can also damage the endothelium to which they adhere, and, if so, by what mechanism.

Employing cultured human endothelial cells as a target tissue for the study of the injurious potential of complement-stimulated PMN, we have documented that the combination of complement-activated serum and PMN, but not either alone, will release excessive $^{51}$Cr from labeled endothelial cells. Moreover, stimulated by our previous demonstration that C5a seems to be the critical activated C component promoting PMN adherence in vitro and pulmonary leukostasis when infused in vivo, we have now demonstrated that purified C5a (but not other low molecular weight chemotactically active fractions) also triggers PMN to damage cultured endothelial cells as well.

Some possible difficulties with our experimental technique require discussion. Thus, we acknowledge that, although statistically significant, the amount of $^{51}$Cr released in these studies is small; furthermore, we recognize that the potential biologic variability between endothelial cells explanted from different umbilical cords might be a potential problem. To address the second difficulty, we devised our experiments to compare chromium release from experimental plates to that from control cultures derived from the same umbilical cord. We have also attempted to address the inherent difficulties of using chromium release as a marker of cellular damage, including the facts that its leakage is usually indicative only of extensive membrane damage, and sublethal injury to cells frequently goes undetected (26). Thus, we utilized in ancillary studies, an unrelated cytotoxicity assay—that of fluorochromasia—to validate endothelial damage. With this test, PMN stimulated by whole activated complement or by C5a were shown to induce a 20–30% reduction in endothelial cell viability, compared to the smaller 5–10% damage suggested by the $^{51}$Cr release assay. Finally, we would emphasize that our experimental model is a static one; it seems likely that endothelial damage might be enhanced if continuous infusion of fresh activated PMN to our culture plates could be effected, so as to more closely reproduce the events which presumably occur during ongoing C activation in vivo.

The principal observation of these studies—that C-stimulated granulocytes damage endothelial cells—suggested initially that a significant effector of this injury might be lysosomal enzymes released as in the "frustrated phagocytosis" model (27–29). In this model, granulocytes that encounter immune complexes or aggregated immunoglobulin deposited on solid surfaces adhere to, and selectively release their lysosomal constituents thereupon, by a process of reverse endocytosis. Indeed, in the one situation in which opsonized particles were layered on our endothelial monolayers, significant lysosomal enzyme (myeloperoxidase) was released from the triggered granulocytes (Table IV). However, such release proved to be the exception, since there was only marginal release of myeloperoxidase from PMN triggered by activated whole serum complement or C5a despite the significant endothelial damage these combinations provoked. Moreover, no correlation between the degree of myeloperoxidase release and the amount of endothelial damage was observed in these studies. The failure to detect myeloperoxidase and lysozyme release supports our view that lysosomal proteases are not primarily responsible for the observed $^{51}$Cr release. It is possible, however, that other (unmeasured) lysosomal enzymes, leukocyte cationic proteins (30), or the myeloperoxidase-$H_2O_2$-halide system (31) could contribute to the observed endothelial cell cytotoxicity.

This proviso notwithstanding, of probably greater importance are toxic oxygen radicals (superoxide anions, free hydroxyl radicals, singlet oxygen), and hydrogen peroxide (13–15, 20); these, as shown by others (19, 21), are generated by PMN provoked by various immune stimuli, including complement and C5a. Such free radicals have been shown to mediate the damage to biomembranes produced by ionizing radiation and oxidative enzymes (32–36), and our results demonstrate they may also damage endothelium. Thus, the combination of xanthine/xanthine oxidase, a well-established, free radical-generating system (37–39), quadrupled chromium release from exposed endothelial cells, and this damage was prevented by the addition to the reaction mixture of superoxide dismutase and catalase—enzymes which enhance the dissipation

of superoxide anions and hydrogen peroxide. Furthermore, this enzyme combination, when added to the buffer bathing endothelial cells, completely blocked activated C/PMN-mediated damage as well. Of further interest, stimulated granulocytes obtained from a single patient with chronic granulomatous disease were 50% less effective in inducing endothelial cytotoxicity than normal granulocytes incubated in parallel. This observation provides additional evidence for the role of free radicals in causing cytotoxicity.

We conclude that these data support our hypothesis that one or more toxic oxygen species play a primary role in complement-directed, leukocyte-mediated endothelial damage. Moreover, since superoxide and other free radicals are generally rapidly dissipated (17), it seems likely that a close physical interdigitation between PMN and endothelium would be necessary for significant cellular damage to occur by this mechanism. This hypothesis was supported, albeit not rigorously proven, by studies in which granulocytes were treated with cytochalasin B. This maneuver was chosen for two reasons. First, cytochalasin will cause PMN that have been previously induced to spread out over millipore filters to round up and retract their membranous veils and pseudopods (25); their adherence to endothelial cells would presumably also diminish thereby. Secondly, cytochalasin-treated PMN, when exposed to C5a or other immune stimuli, have an unimpaired capacity to release superoxide anions (19). Thus, it was anticipated that cytochalasin would reduce close physical contact between PMN and endothelium without interfering with the free radical-generating capacity of PMN. The fact that cytochalasin treatment markedly inhibited endothelial damage (Fig. 2) suggests that close proximity between effector and target cell may, indeed, be required in this experimental system.

One question which we have thus far not addressed regards the specificity of endothelial cell vulnerability to complement-activated PMN. We have not assayed damage induced by this kind of stress to any other target cells (e.g. fibroblasts), although we consider the presented data of more intrinsic interest, because of the unique front-line exposure of endothelium to "angry" white cells. In this regard, we believe our results may have relevance to the understanding of endothelial damage in diverse clinical syndromes. For instance, shock lung, a condition characterized by high-protein pulmonary edema, results from pulmonary endothelial injury, and is frequently associated with C-activating insults such as gram-negative bacteremia. Also, although direct evidence is not yet available, we speculate that the accelerated atherosclerosis seen in chronically hemodialyzed patients might reflect the frequent activation of C by dialyzer cellophane, with resulting PMN-mediated endothelial damage.

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