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Mechanism of Anti-Complementary Activity of Corticosteroids *in Vivo*: Possible Relevance in Endotoxin Shock (39638)

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Introduction. Corticosteroids protect animals from potentially lethal doses of endotoxin (1-6), but the mechanism for this protection remains as controversial as the mechanism for the toxicity of endotoxin itself. Recent evidence suggests that complement activation may be involved because: (a) Endotoxin added to serum *in vitro* activates complement predominantly through the alternative pathway (7); (b) patients in endotoxinemic shock manifest depletion of alternative pathway components (8); and (c) dogs (9) or rabbits (10) depleted of complement are protected from usually lethal doses of endotoxin. The possibility that corticosteroids may inhibit the deleterious effects of endotoxin by affecting the complement system is an attractive one. *In vitro*, corticosteroids depress the functional and immunochemical titers of the first four components of the complement cascade (11, 12), and, thereby, inhibit complement-mediated red cell lysis (11), immune adherence (11), and mast cell histamine release (13). Furthermore, since neutropenia commonly occurs with endotoxinemia, the recent observation that neutropenia is constantly associated with *in vivo* complement activation (14) lends further credence to the suggestion that complement activation may be critical in endotoxin toxicity. Thus, McCall *et al.* (14) have shown that *in vivo* activation of complement by intravascular injections into rabbits of cobra venom factor (CVF) or inulin, or, alternatively, infusion of plasma in which complement is preactivated by incubation with either agent, induces a profound transient neutropenia followed by neutrophilia. The neutropenia-inducing substance (NIS) was found to be a low molecular weight (<20,000 daltons) moiety formed when complement is activated by CVF or inulin.

Studies from our laboratory have expanded these observations (15) by demonstrating that the transient profound neutropenia seen in uremic patients shortly after institution of hemodialysis is caused by an analogous activation of complement which occurs when blood is pumped over the polycellulose of dialysis coil membranes. Moreover, we have shown that simple incubation of plasma with dialysis coil cellophane (or inulin or zymosan) generates a heat-stable substance, which, upon infusion into rabbit veins, induces transient neutropenia due to pulmonary vascular sequestration of neutrophils. The dialysis membrane activates complement predominantly through the alternative pathway (15), much as do the other polysaccharides, inulin and zymosan (7), but offers the advantage of being a nonsoluble, easily removable, and sterilizable complement activator.

In the present studies we have evaluated the mechanism by which corticosteroids may affect one complement-mediated phenomenon, the generation of NIS. We have done so by infusing autologous plasma incubated with dialysis membrane or inulin as a model of *in vitro* generation of NIS, and, alternatively, by infusing CVF as a model of its *in vivo* formation. Our results demonstrate that corticosteroids inhibit the formation of NIS during complement activation, but do not alter the effects of this substance once formed. Interestingly, the dose relationships and kinetics of this corticosteroid inhibition mimic exactly those previously demonstrated in endotoxin-protection experiments, suggesting that certain complement-inhibiting effects of corticosteroids, particularly their ability to inhibit NIS generation, may underlie their protective role in endotoxemia.

Materials and methods. For *in vitro* complement-activation studies, blood was withdrawn from a femoral artery of 2-4-kg al-

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bino white rabbits, after which it was quickly chilled to 0° and centrifuged. Plasma was removed, heparinized (1 U/ml), and diluted by 10% with either sterile normal saline or a corticosteroid dissolved in saline. The diluted plasma was incubated at 37° for 30 min and then exposed to either polycellulose dialysis membrane (20 cm^2/ml) or inulin (0.2 mg/ml) for another 30 min at 37°. The preparation was freed of inulin by centrifugation at 10,000 rpm, or of dialysis membrane by manual removal, and then reinfused into the fermoral vein of the original donor animal over a 30-sec period. At various times thereafter, samples were taken from a femoral artery, and neutrophil counts were compared with those obtained in samples taken less than 30 sec prior to infusion. In studies of *in vivo* complement activation, CVF, purified and assayed by the method of Cochrane *et al.* (16), was infused in a dose of 40 U/kg into the femoral vein of 12 untreated control rabbits or into corticosteroid-pretreated rabbits (two rabbits at each dose). Blood samples were removed and analyzed as above. Hydrocortisone 21-sodium succinate (Solu-Cortef), 6α-methylprednisolone 21-sodium succinate (Solu-Medrol), and dexamethasone (Decadron) were diluted to appropriate concentrations in sterile normal saline and adjusted to pH 7.4 prior to incubation with plasma. Some animals were infused over 5-min intravenously with such prepared corticosteroids prior to CVF infusion.

Results. Autologous plasma pre-exposed to the complement activator, cellophane (or inulin, not shown) rapidly induced reproducible transient neutropenia when reinfused; the degree of neutropenia was proportional to the quantity of plasma reinfused (solid line, Fig. 1). The nadir of neutropenia developed within 3 min, and neutrophilia occurred regularly in 10–30 min; moreover, untreated plasma or plasma voided of activatable complement by preheating (56°) or hydrazine treatment lacked NIS activity when reinfused (not shown). Preincubation of plasma with corticosteroids prior to polycellulose membrane exposure inhibits the neutropenia and does so in a dose-related fashion; that is, the greater the amount of activated plasma injected, the greater the level of corticosteroid needed for inhibition (interrupted lines, Fig. 1). Moreover, the concentrations of corticosteroid required for inhibition are in the same range as those previously reported as inhibiting complement-mediated red cell lysis (11), immune adherence (11), and mast cell histamine release (12) *in vitro*; as with other corticosteroid effects, Solu-Medrol is more potent than Solu-Cortef. Infusion of corticosteroids alone induced minimal neutrophilia resembling the upper two lines of Fig. 1.

In marked contrast, if corticosteroids are incubated with plasma after complement has already been activated by incubation with cellophane (or inulin, not shown), inhibition of neutropenia does not occur (Table I). Analogously, if animals are treated directly with corticosteroids (5–250 mg/kg of Solu-Cortef of Solu-Medrol) before infusion of complement-activated plasma, they develop neutropenia identical to that noted in untreated animals (Table I). These results demonstrate that corticosteroids inhibit the fluid phase formation of NIS, but, once this factor is formed, they have no effect on its action *in vivo*. Although only data utilizing cellophane-activated plasma are shown, identical results were obtained with infusions of plasma exposed to inulin.

Activation of complement *in vivo* by CVF infusion is also associated with neutropenia

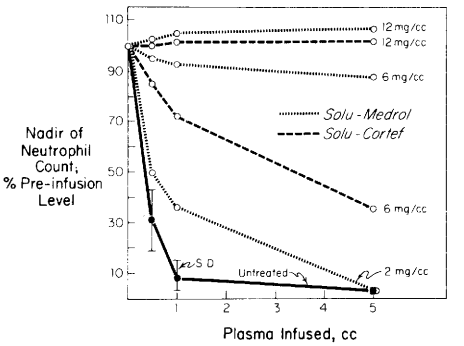

FIG. 1. Inhibition of C-induced neutropenia by corticosteroids *in vitro*. Infusion of increasing amounts of autologous plasma in which complement components had been activated by preincubation with dialysis-coil cellophane produces progressively severe neutropenia in rabbits (solid line). If corticosteroids are added prior to cellophane exposure, a dose-related inhibition of the neutropenia effect results (interrupted lines).

TABLE I. LACK OF EFFECT ON NEUTROPENIA WHEN CORTICOSTEROIDS ARE USED AFTER COMPLEMENT ACTIVATION.

Infused plasma (ml)	Nadir of neutropenia (percentage of baseline) when		
	Untreated (N = 8)	Steroid added after C activation[a]	Steroid treated[b]
0.75	31.5 ± 5.6[c]	27	24.1 ± 9.3
1.00	8.4 ± 2.2	3.8 ± .2	6.2 ± 1.0
5.00	3.3 ± 0.8	2.0	3.9 ± 3.8

[a] Solu-Medrol (12 mg/ml) added to plasma after its treatment with dialysis membrane. Five animals were studied at the 1.00-ml dose and two animals each at the 0.75- and 5.00-ml doses of plasma.

[b] Solu-Medrol (60 mg/kg) infused with rabbit 10 min prior to complement-activated plasma infusion. Five animals were studied in each group.

[c] SE.

which lasts 30–60 min (solid line, Fig. 2). Neutropenia is inhibited by pretreating animals with Solu-Medrol in a dose-related manner (interrupted lines, Fig. 2); Solu-Cortef is similarly and appropriately effective. In striking contrast, Decadron is less effective, relative to its usual 20–30 and 5–8 times greater relative potency compared to Solu-Cortef and Solu-Medrol. Thus, minimally inhibitory doses of Solu-Cortef and Solu-Medrol given 10 min prior to CVF are 100 and 20 mg/kg, respectively; the expected corresponding dose of Decadron should be approximately 3.75 mg; instead, at least 15 mg/kg was required to inhibit neutropenia (not shown).

When corticosteroids are given at increasingly remote times before CVF injection, their effects are progressively lessened. For instance, a well-marked inhibitory effect of a given dose of Solu-Cortef administered 10 min before CVF becomes less effective if administered 30 min before, and is totally without effect if administered 90 min before the complement activator (Fig. 3). Moreover, if the dose of infused CVF is doubled (to 80 U/kg) a comparable increase in injected corticosteroid is required to significantly inhibit the induced neutropenia (not shown).

Discussion. These results allow two conclusions. (a) Corticosteroid inhibition of NIS formation appears to be reversible and related to the concentration of circulating corticosteroid and to the strength of complement activator injected. (b) Decadron is relatively impotent in preventing NIS generation; this relative impotence has also been noted in recent studies which compared the ability of various corticosteroids to inhibit neutrophil accumulation in inflammatory exudates (17), an accumulation which others have demonstrated to be dependent on complement activation (18).

The results of these *in vivo* experiments are also strikingly similar to those noted by others when corticosteroids were used to protect animals form endotoxin. Thus, high steroid doses are protective, and lower

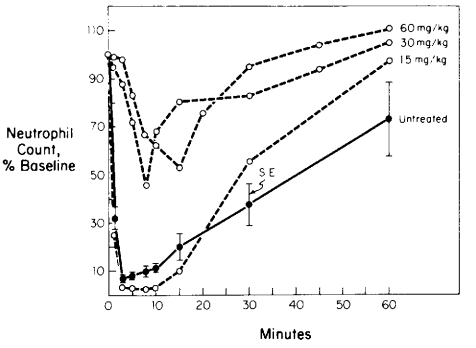

FIG. 2. Dose-related inhibition of CVF-induced neutropenia by corticosteroids *in vivo*. Rapid neutropenia occurs in rabbits in which complement is activated by administration, at 0 time, of purified cobra venom factor (solid line); an effect inhibited by high, but not low, doses of Solu-Medrol given 10 min before the CVF (interrupted lines). Solu-Cortef, but not Decadron, was also appropriately inhibitory (not shown).

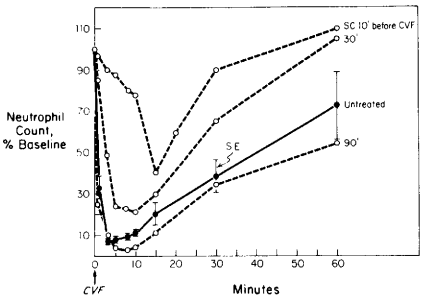

FIG. 3. Diminished activity of Solu-Cortef (SC) when administered at intervals remote from CVF. Although the usual neutropenia induced by CVF injection into rabbits (solid line) is significantly ameliorated by Solu-Cortef (150 mg/kg) given 10 min before CVF (top line), its inhibitory effect is progressively blunted when given at more remote times.

doses are much less so (1, 5, 6). When 2700 mice were given intraperitoneally simultaneous lethal doses of endotoxin and corticosteroids, the doses of Solu-Cortef, Solu-Medrol, and Decadron needed to halve mortality were 146, 70.9, and 18.6 mg, respectively (1); that is, Decadron was relatively impotent. Furthermore, corticosteroids given 0.5–1 hr prior to endotoxin were less protective than when given simultaneously (5, 6), and, if given 2 hr prior, they were not protective at all (6). As with the CVF studies in the present report, if the amount of infused endotoxin was increased, the protection afforded by a given dose of corticosteroid was offset (5, 6). Taken together, these data suggest that corticosteroid protection in endotoxinemia is also a reversible one related to the dose of corticosteroid and the amount of endotoxin injected, and that Decadron is relatively ineffective.

From these similarities, we suggest that complement-associated generation of NIS may be involved in endotoxin shock. Furthermore, our results indicate that, once NIS is formed, corticosteroids are ineffective in altering its effects. However, inhibition of the further generation of this potentially deleterious substance is possible, but only with very high concentrations of particularly two of these drugs, Solu-Cortef and Solu-Medrol; Decadron is less useful. In this regard, it is of interest that previous studies of the efficacy of corticosteroids in ameliorating human endotoxin shock have been conflicting. If NIS generation is indeed critical in endotoxin shock, most of these studies would not have used doses of corticosteroids sufficient to block such generation. From the results presented, we suggest that very large doses (perhaps as high as 30 mg/kg of Solu-Medrol or 150 mg/kg of Solu-Cortef) and frequent administration of these agents may prove beneficial in human endotoxin shock, and studies employing such regimens should be underaken.

Summary. The effects of several corticosteroids on the sudden neutropenia which occurs in animals exposed to activated complement components were studied. Solu-Medrol and Solu-Cortef in high concentrations inhibited such neutropenia but only if present before or during the period of complement activation; Decadron was much less efficient. The observed dose and temporal relationships are strikingly similar to those noted in previous studies of corticosteroid protection from endotoxin shock, suggesting that complement-mediated alterations in neutrophils may be critical in this entity.

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