Polymorphonuclear Leukocyte Adherence to Nylon: Effect of Oral Corticosteroids

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The effect of orally administered glucocorticoids on polymorphonuclear leukocyte adherence was studied by using the nylon fiber adherence assay. Inhibition of adherence 4 h after administration of the agent was confirmed, and in addition, augmentation of adherence was noted 24 h after ingestion of prednisone. Cross-over studies revealed inhibition and augmentation to be cell associated as well as plasma mediated. Suspension of washed, dextran-sedimented polymorphonuclear leukocytes, harvested 4 h after prednisone ingestion in base-line or 24-h plasma failed to reverse inhibition of adherence. Adherence of 24-h polymorphonuclear leukocytes was augmented when suspended in all test plasmas. Plasma-mediated effects were demonstrated by inhibition of base-line adherence of polymorphonuclear leukocytes suspended in 4-h plasma and augmentation of adherence of cells in 24-h plasma. Plasma-mediated effects were reversible by washing.

The effect of glucocorticoids upon polymorphonuclear leukocyte (PMN) number and function has been investigated extensively to determine both the mechanism of increased susceptibility to infection (2) and the benefits of steroid therapy in specific clinical situations such as dialysis leukopenia and bacteremic shock (19). Release from the bone marrow reserve (6) and decrease in PMN adherence to nylon (4, 14) after administration of corticosteroids have been reported. Migration into skin windows or inflammatory exudates is also depressed by steroid therapy (7). Hydrocortisone when added in vitro in high concentrations to PMN suspensions reduces the oxidative burst which accompanies phagocytosis and interferes with killing of Staphylococcus aureus (17). PMN obtained from patients receiving the anti-inflammatory glucocorticoids demonstrate inhibited reduction of nitroblue tetrazolium (3).

The present study was undertaken to investigate the effect of oral administration of corticosteroids upon PMN adherence to nylon, a phenomenon analogous to the sticking of PMN to endothelium (15). The nylon adherence assay has facilitated these investigations because it is easy to perform (14), reproducible (18), and altered by inflammation and anti-inflammatory agents (12, 16).

MATERIALS AND METHODS

Subjects. Normal male volunteers aged 23 to 54 were asked to participate in the study. Blood was drawn before and at various intervals after administration of oral doses of prednisone (Deltasone, Upjohn, Kalamazoo, Mich.). Each dose of prednisone was administered at least twice to each volunteer to obtain the appropriate plasma and PMNs. The study was fully explained to the volunteers based on procedures approved by the Institutional Review Board at Northwestern University.

PMN preparation. Blood was drawn into sterile, heparinized syringes to produce a final heparin concentration of 7.5 μg of blood per ml. For studies requiring separated PMNs, venous blood was mixed with 6% dextran and saline (type 200, lot 41C, 1546; Sigma Chemical Co., St. Louis, Mo., placed in sterile plastic tubes, and allowed to stand for 45 min at room temperature to sediment the erythrocytes. The leukocyte-rich supernatant was centrifuged at 180 × g for 5 min. The cell button was washed twice in Hanks balanced salt solution (HBSS) with 1% gelatin and heparin (10 μg/ml). The cell concentration was then adjusted to achieve 10,000 to 20,000 PMNs/mm³ in 2 ml of HBSS. Subsequently an additional 2 ml of autologous plasma or HBSS was added to bring the cell suspension to a final concentration of 5,000 to 10,000 cells/mm³ (18).

Plasma and serum preparation. Plasma was obtained from heparinized whole blood, centrifuged at 750 × g for 15 min. The plasma was decanted from the cells and used immediately or frozen at –70°C. Serum was obtained from whole blood which had been allowed to clot at 37°C for 60 min. It was used immediately or frozen at –70°C until needed for experiments.

Determination of adherence. PMN adherence was measured by the method of MacGregor et al. (14) as previously reported by this laboratory (18). Scrubbed nylon fiber (3 denier, 3.81 cm, type 200; Fenwal Laboratory, Deerfield, Ill.) columns were constructed of specific height (15 mm) and weight (80 or
50 mg) in Pasteur pipettes. A 1-ml amount of whole blood or PMNs suspended in plasma was pipetted over each of three columns of nylon. A hemocytometer and standard Wright stain were utilized to count the PMNs in the original and effluent sample. The percentage of PMNs adherent to the nylon was then calculated. For dextran-sedimented PMNs, the differential was done in the chamber; only cells with three nuclear lobes were designated as granulocytes. The values for each of the three columns were averaged to determine the adherence value.

Cross-over studies. Cross-over studies were performed by using PMNs and autologous plasma obtained from normal volunteers. Volunteers received 10 and 40 mg of prednisone; one also received 25, 50, 67.5, and 75 mg. Each volunteer took, on 1 day of consecutive weeks, the specified amount of prednisone. Before and at 90 min, 4, 24, and 48 h after the administration of the corticosteroid, plasma and PMNs were harvested, permitting the study of PMNs after suspension and incubation in all possible plasma. The PMNs were assayed immediately, after 30 min of incubation in a specific plasma or after a 30-min incubation, subsequent wash, and suspension in HBSS.

Statistical analysis. Results were analyzed by using the Student t test.

RESULTS

Initial studies, undertaken with heparinized whole blood obtained from a single volunteer, evaluated granulocyte adherence over time after administration of increasing doses of prednisone (25, 50, and 75 mg). Adherence was measured before and at 1, 2, and 4 h after prednisone. Mean adherence values fell from 92.6% before prednisone, to 83% at 1 h, to 59% at 2 h, and to a minimum of 39.3% at 4 h after administration of the agent.

Adherence assays using whole blood obtained from five normal volunteers before and after prednisone administration confirmed the finding of inhibition of adherence at 4 h. In addition, 24 h after ingestion of prednisone, augmentation of PMN adherence was noted. Normal adherence, equal to base-line values, was found at 48 h. Inhibition was noted after administration of high-dose (40 mg or more) or low-dose (10 mg) prednisone orally and was more clearly demonstrated with the 80-mg column (Table 1). The 60-mg column of nylon fiber enhanced demonstration of augmented adherence of PMNs in whole blood harvested at 24 h. Mean 24-h adherence values were 128.6 and 139.6% of base line, using the lower weight of fiber, in contrast to 112 and 103.4% noted with the 80-mg column. Cross-over experiments were conducted by using dextran-separated and washed PMNs suspended in autologous plasma as outlined in Materials and Methods. These experiments were undertaken to determine whether the inhibition and augmentation of PMN adherence noted by whole blood were cell associated or plasma mediated and to obviate the problem of leukocytosis induced by steroid administration. Cells harvested 4 h after steroid administration were inhibited when suspended in all plasmas. In contrast, PMNs obtained 24 h after corticosteroid ingestion demonstrated enhanced adherence in all plasmas. Plasma obtained 4 and 24 h after prednisone inhibited and augmented adherence of base-line cells significantly. Plasma harvested at 4 h also significantly lowered adherence of 24-h PMNs (Fig. 1) but 4-h cells were less affected by 24-h plasma. The plasma-mediated effects required incubation of the PMNs in the specific plasma for 30 min at 37°C and were reversed by washing (Table 2).

To further delineate plasma from cell-associated effects, cross-over experiments were carried out by using base-line, 90-min and 4-h plasma and base-line, 90-min and 4-h PMNs. Marked inhibition of adherence of 4-h PMNs (12.9%) in comparison with base-line PMNs (58.2%) or 90-min PMNs (56.1%) was noted when cells were suspended in base-line plasma. In contrast, 90-min and 4-h plasma inhibited adherence of base-line PMNs equally well (Table 3).

The inhibitory and augmenting effects of serum were compared with those of plasma. Serum and plasma harvested 4 and 24 h after ingestion of the corticosteroid were incubated with normal cells. Serum or plasma obtained at 4 h inhibited

<table>
<thead>
<tr>
<th>Fiber wt (mg of nylon)</th>
<th>Dose*</th>
<th>Zero time</th>
<th>Adherence at:*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 h</td>
<td>24 h</td>
</tr>
<tr>
<td>60</td>
<td>High</td>
<td>62.1 ± 8.5</td>
<td>42.2 ± 3.7, P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>56.2 ± 3.2</td>
<td>49.2 ± 10.6, NS</td>
</tr>
<tr>
<td>80</td>
<td>High</td>
<td>71.0 ± 5.7</td>
<td>44.4 ± 8.3, P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>76.1 ± 9.0</td>
<td>63.3 ± 11.5, P &lt; 0.05</td>
</tr>
</tbody>
</table>

* Each adherence value indicates mean ± standard deviation of 12 assays. See text for details. NS, Not significant.

* High dose was 40 or 67 mg of prednisone. Low dose was 10 mg of prednisone.
adherence of base-line cells (86.7 and 82.8% of adherence in base-line serum and plasma, respectively). However, 24-h serum, in contrast to 24-h plasma, did not augment adherence of base line in PMNs (102 versus 130% of base line).

**DISCUSSION**

Craddock et al. have reported that incubation of plasma with nylon does not alter measurable C3 but does induce an increase in C5α. Plasma treated in this manner produces leukocyte aggregation in vitro and neutropenia (5). Previous studies from our laboratory, however, have demonstrated that base-line adherence of granulocytes to nylon is not a result of filtration of PMN aggregates induced by nylon activation of complement and that base-line adherence occurs in the absence of plasma (18). Reports have indicated that oral administration of prednisone produces inhibition of adherence of PMN to nylon (4, 14, 15). The present studies confirm and extend these findings. After oral administration of prednisone a fall in adherence values is noted. However, by 24 h, cells appear to be increasingly adhesive and adherence values are significantly increased above the base line. These findings were confirmed in studies of 10 asthmatic patients receiving alternate-day prednisone (J. Chiang, R. Patterson, and J. McGillen et al., submitted for publication).

Inhibition was demonstrated after administration of 40 and 10 mg of prednisone by using the 80-mg columns. Demonstration of augmented adherence required the 60-mg column. This finding agrees with previous reports of enhanced adherence of PMNs obtained from patients with inflammatory disease states, using lower weights of nylon (16).

Cross-over experiments using dextran-separated, washed PMNs support the hypothesis that inhibition induced by oral prednisone represents both cell-associated as well as plasma-dependent effects. However, maximal modifica-

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**TABLE 2. PMN adherence: effects of incubation of PMN with autologous plasma and washing**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>PMN adherence at the following incubation times:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 s</td>
</tr>
<tr>
<td>4 h</td>
<td>38.2 ± 7.7</td>
</tr>
<tr>
<td>24 h</td>
<td>54.5 ± 4.1</td>
</tr>
</tbody>
</table>

* PMNs were obtained by dextran sedimentation before prednisone administration (40 mg).
* Plasma was obtained 4 and 24 h after prednisone administration (40 mg).
* PMNs obtained by dextran sedimentation.

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**TABLE 3. Effect of incubation of autologous PMNs in autologous plasma after prednisone administration (40 mg): adherence values**

<table>
<thead>
<tr>
<th>Plasma</th>
<th>PMNs at the following times after prednisone:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
</tr>
<tr>
<td>Base line</td>
<td>58.2 ± 5.5</td>
</tr>
<tr>
<td>90 min</td>
<td>43.8 ± 8.1</td>
</tr>
<tr>
<td>4 h</td>
<td>41.5 ± 14.3</td>
</tr>
</tbody>
</table>

* Each adherence value represents mean ± standard deviation for nine assays. See text for details.
* Plasma was obtained before and at 90 min and 4 h after prednisone administration.
* PMNs obtained by dextran sedimentation.

FIG. 1. Cell and plasma contribution to inhibition and augmentation. Cell-associated and plasma-mediated alteration of PMN adherence after oral administration of 40 mg of prednisone to four volunteers. The first three bars present adherence values of normal PMNs suspended in autologous plasma harvested before and at 4 and 24 h after prednisone. Adherence values of 4- and 24-h cells in the three plasmas are depicted in the second and third sets of bars. Adherence of 4-h cells is significantly inhibited, and 24-h cells are augmented when PMNs are suspended in any of the three autologous plasmas. Adherence of base-line cells is significantly inhibited by 4-h plasma and augmented by 24-h plasma. The augmentation of adherence of 24-h PMNs was decreased by suspension in 4-h plasma. Bars represent the mean ± standard deviation of 12 adherence assays.
tion of PMN adherence is distinct in time from the maximal plasma-induced alteration. At 90 min, coincident with peak plasma levels of the corticosteroid, maximal plasma-mediated inhibition is noted which persists for at least 4 h (adherence = 43.5% versus 58%). In contrast, minimal cell-associated alterations are noted at 90 min (55.1 versus 58%), and maximum inhibition (12.9%) occurs 4 h after oral corticosteroid administration. The inhibition of adherence of base-line PMNs induced by 90-min and 4-h plasma and the augmentation of adherence noted with suspension of PMNs in 24-h plasma require incubation of the cells in the plasma and can be reversed by washing.

The mechanism of inhibition of adherence subsequent to corticosteroid administration is unclear. Inhibition coincides with a maximum rise in granulocyte number produced by glucocorticoid administration (6). The decrease in adhesiveness of PMNs may reflect an increase in the number of immature, possibly less adherent cells released from marrow into the circulating pool (13). A return to normal adherence values could represent a return to the usual distribution of PMNs among the circulating, marginal, and marrow pools (1). Studies of Klemppner and Gallin have demonstrated that peripheral PMNs are not homogeneous. Eighty percent form rosettes with immunoglobulin G-coated erythrocytes. Rosettes forming PMNs are more adherent to nylon, phagocytize, and kill S. aureus more efficiently and are more responsive to chemotactic stimuli (10). Addition of hydrocortisone in vitro to PMN suspensions interferes with the binding of membrane Fc receptor and immunoglobulin G-coated erythrocytes (11). In vitro alteration in PMN membranes by steroids therefore may explain the decrease in adherence noted in cells harvested 4 h after the prednisone ingestion but offers no insight into the mechanism underlying augmentation noted at 24 h.

These results demonstrate a cell-associated in addition to a plasma-mediated corticosteroid effect. MacGregor et al. reported a plasma effect only; this disparity may be explained by differences in cell separation techniques or incomplete removal of a humoral inhibitory factor by washing of dextran-separated PMNs. However, vigorous washing of PMNs can induce augmented adhesiveness (20) independent of other manipulations.

The enhanced adhesiveness of PMNs obtained 24 h after prednisone administration was also cell associated as well as plasma mediated. Augmentation of PMN adherence has been noted in assays of whole blood obtained from patients with inflammatory disease (15). This enhancement appears to be plasma but not serum dependent as suspensions of ABO-compatible PMNs in plasma, but not serum, obtained from patients increased adherence. The plasma factor was heat stable and demonstrable in the presence of guinea pig complement. The addition of fibrinogen to serum did not restore this augmenting factor (16). However, cell-associated augmentation was noted with rabbit peritoneal exudate PMNs (12), suggesting an irreversible cell-associated change analogous to C5a-induced membrane effects (9). The augmentation of 24-h PMN adherence after prednisone is cell associated and not reversed by washing and suspension of 24-h cells in base-line or 4-h plasma. This cell-associated augmentation may be due to irreversible binding of plasma factor(s), alteration in the PMN membrane independent of humoral effects, or a shift in human neutrophil subpopulations (6). In contrast to our findings, Clark and co-workers have recently described partial return to normal adherence values, not augmentation 24 h after administration of 60 mg of prednisone. Reasons for the disparity in results are unclear. Although their technique for measuring adherence differed, a lower weight of nylon fiber was packed into a smaller volume than that in the present experiments (4).

PMN adherence to nylon, which is analogous to PMN adherence to endothelial cells, represents an in vitro correlate of the first step required for migration of PMN into inflammatory foci (15). Adherence is inhibited by oral administration of prednisone. Inhibition of adherence may represent a major defect underlying the increased susceptibility to pyogenic infection noted in patients receiving daily corticosteroids. Conversely, the augmented and normal values noted at 24 and 48 h after glucocorticoid ingestion may account for the lack of susceptibility when steroids are administered on an alternate-day schedule (7).

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LITERATURE CITED


