Ally, A. I., Nakatsu, K. (1976) J. Pharmacol. Exp. Ther. 199: 208–215

Burnstock, G. (1972) Pharmacol. Rev. 24: 509–581

Burnstock, G., Campbell, G., Satchell, D., Smythe, A. (1970) Br. J. Pharmacol. 40: 668–688

Jahnberg, T. (1977) Scand. J. Gastroenterol. 12: suppl. 46

Lefebvre, R. A., Willems, J. L. (1978) Arch. Int. Pharmacodyn. Ther. 234: 342–343

Okwuasaba, F. K., Hamilton, J. T., Cook, M. A. (1977) Eur. J. Pharmacol. 46: 181–198

Reyntjens, A. J., Niemegeers, C. J. E., Van Nuetten, J. M., Laduron, P., Heykants, J., Schellekens, K. H. L., Marsboom, R., Jageneau, A., Broekaert, A., Janssen, P. A. J. (1978) Arzneim-Forsch. 28: 1194–1196

Valenzuela, J. E. (1976) Gastroenterology 71: 766–769

Wang, S. C., Borison, H. L. (1952) Ibid. 22: 1–12

Effects of hydrocortisone on binding of IgG or C3b-coated erythrocytes to human monocytes and polymorphonuclear leucocytes

G. TOLONE*, L. BONASERA, R. SAJEVA, *Istituto di Patologia Generale, Università di Palermo, Corso Tukory, 211, 90134-Palermo, Italy*

Over the past decade, studies in many laboratories have suggested that anti-inflammatory steroids are capable of inhibiting each of the steps in the phagocyte-foreign body interaction, thereby rendering the host more susceptible to aggression by microorganisms (Katler & Weissmann 1977). These results, if extended and confirmed, would adequately account for the increased incidence of infection in patients treated with steroids for long periods of time. However, in most studies steroid concentrations were higher than those used therapeutically, thus making it difficult to evaluate the pharmacological relevance of the experimental findings. Furthermore, conflicting results have been obtained probably because of differences in test systems, time of incubation or steroid dosage. Thus, while Rinehart et al (1974) have claimed that corticosteroids have no effect on binding of IgG or C3b-coated erythrocytes to human monocytes, Schreiber et al (1975) have reported that several steroids, including hydrocortisone, inhibit monocyte receptor activity for both IgG and C3b in a dose-response fashion. We have therefore re-examined the effects of hydrocortisone on monocyte receptor activity and have extended the investigation to include polymorphonuclear leucocytes.

Monocytes and polymorphonuclear leucocytes (PMN) were isolated from human peripheral blood according to Ehlenberger & Nussenzweig (1977). Isolated monocytes were resuspended in 3 ml of medium (RPMI 1640 with added 10 mM Hepes, pH 7·4; GIBCO) containing 15% autologous serum. 100-μl aliquots of this suspension (1×10^6 cells ml^{-1}) were layered on glass cover slips (10×10 mm) and these were incubated for 30 min at 37 °C under a 5% CO_2 atmosphere. Non adherent cells and serum were decanted and the adherent cells were washed four times with medium, incubated for 30 min at 37 °C in fresh medium and washed once more before being used in the binding assays. Monolayers were more than 95% mononuclear, and 99% of these cells were viable. Isolated PMN were resuspended in plasma (1×10^8 cells ml^{-1}) and 200 μl aliquots of the suspension were layered on cover glasses (18×18 mm) and incubated for 20 min as above. Monolayers were washed gently by dipping the cover slips in three successive beakers of fresh medium, and then incubated (30 min at 37 °C) in Petri dishes filled with medium supplemented with 15% foetal calf serum. They were rinsed in medium once more before use in binding assays. Monolayers were usually 90% polymorphonuclear, and 97% or more of these cells were viable. Sheep red blood cells (E) in Alsever's solution (10^9 cells ml^{-1}) were ^{51}Cr-labelled (Tolone 1968) and sensitized with an appropriate dilution of rabbit IgM anti-E (Cordis). After washing, IgM-sensitized cells (EA) were coated with C3b via sequential addition of purified C1, C4, C2 and C3 (Cordis). Coated cells were washed in medium containing 10 mM Na_3HEDTA to remove C1, and incubated at 37 °C for 2 h to decay C2. These cells are referred to as EAC3b although they also contain C4b. Aliquots of either EA or EAC3b were coated with IgG by incubating (30 min at 37 °C) 1 ml volumes of each preparation (10^9 cells) with equal volumes of medium containing an appropriate dilution of rabbit IgG anti-E (Cordis). These cells are referred to as EAIgG and EAC3bIgG respectively. The number of C3b or IgG molecules bound per E (800 and 400 respectively) was determined by C1 fixation and transfer (Schreiber et al 1975). Binding assays were performed by overlaying cover slips (each containing about 5×10^4 phagocytes) with $1{\cdot}25 \times 10^7$ opsonized E in 0·25 ml of medium supplemented (or not) with hydrocortisone 21-sodium succinate (Sigma) at the indicated concentrations, incubating 45–60 min at 37 °C, decanting unbound E and washing five times with fresh medium. Phagocyte monolayers were then lysed with H_2O and the lysate was assayed for radioactivity in a gamma counter. All experiments were in triplicate.

Under our experimental conditions, where a large excess of erythrocytes is allowed to settle on the phagocyte monolayers thereby providing a saturating cell density and absence of shear forces between erythrocytes and phagocytes, both monocytes and PMN-leucocytes bind effectively to IgG or C3b-coated sheep red blood cells (Table 1). This binding is markedly

* Correspondence.

Table 1. Binding of ^{51}Cr-labelled sheep red blood cells coated with IgG or C3b to human monocytes and polymorphonuclear leucocytes.

Erythrocytes coated with	Binding to Monocytes	PMN-leucocytes
IgG	2100	1600
C3b	2900	2000
IgG + C3b	8400	6800

Results are expressed as counts per min specifically bound per monolayer, where the binding of uncoated erythrocytes (EA) was used as a measure of non-specific binding and subtracted from the total counts bound. Values represent the mean of triplicate samples which were ± 10% of the mean.

increased by the presence of the two opsonins on the same erythrocyte surface. In terms of cells bound per phagocyte, coating with both IgG and C3b causes an increase of over 300% in E binding to monocytes or to PMN-leucocytes. That is probably because C3b and IgG act synergistically in opsonization greatly overcoming the electrostatic repulsion which hampers erythrocyte binding to phagocytes (Ehlenberger & Nussenzweig 1977). Thus, the binding of either monocytes or PMN-leucocytes to E sensitized with both IgG and C3b is greater than the additive binding observed with E coated with either IgG or C3b (Table 1). In this in vitro system, hydrocortisone at a concentration of 1×10^{-4} M does not affect the binding of C3b or IgG-coated erythrocytes to human monocytes. Higher doses of the steroid ($5–7{\cdot}5 \times 10^{-4}$ M) are required for inhibition to occur, but, even in these conditions, E coated with both opsonins (C3b and IgG) bind normally to monocytes (Table 2). A somewhat different response to hydrocortisone is found with polymorphonuclear leucocytes. As shown in Table 3, a 40% decrease in EAC3b or EAC3bIgG binding to PMN-leucocytes occurs in samples incubated with $2{\cdot}5 \times 10^{-5}$ M hydrocortisone. Binding of EAC3bIgG is also affected, but to a lesser extent (20% inhibition). As observed for monocytes, PMN-leucocytes adhere more avidly to double opsonized erythrocytes by virtue of the synergistic interaction between IgG and C3b receptor sites (Table 1).

Table 2. Effects of hydrocortisone on human monocyte binding to heterologous erythrocytes coated with IgG, C3b or both.

Hydrocortisone concn (M)	Monocyte binding to (% of control) EAIgG	EAC3b	EAC3bIgG
$1{\cdot}0 \times 10^{-4}$	97	108	102
$2{\cdot}5 \times 10^{-4}$	80	91	100
$5{\cdot}0 \times 10^{-4}$	58	65	95
$7{\cdot}5 \times 10^{-4}$	35	47	88

Values represent the mean of triplicate samples which were ±5% of the mean.

Table 3. Effects of hydrocortisone on human polymorphonuclear leucocyte binding to heterologous erythrocytes coated with IgG, C3b or both.

Hydrocortisone concn (M)	PMN-leucocyte binding to (% of control) EAIgG	EAC3b	EAC3bIgG
$5{\cdot}0 \times 10^{-6}$	95	106	98
$1{\cdot}0 \times 10^{-5}$	78	85	102
$2{\cdot}5 \times 10^{-5}$	56	63	80
$5{\cdot}0 \times 10^{-5}$	30	41	58

Values represent the mean of triplicate samples which were ±5% of the mean.

The present results point to polymorphonuclear leucocytes, rather than monocytes, as a possible target for the inhibitory effects of hydrocortisone on phagocyte receptor activity. Probably because of differences in surface lipid bilayers or in protein membrane receptors, these cells are 20 times more susceptible than monocytes to the action of the steroid. Both polymorphonuclear and mononuclear phagocytes ingest and kill bacteria at sites of inflammation, but PMN-leucocytes are the primary line of defense against bacterial infections, partly because they can be mobilized earlier, and their number in the circulation can be rapidly expanded by the host. It follows that any functional impairment of their activity is expected to reduce the critical number of microorganisms required for the development of an infection. Therefore, it seems reasonable to suggest that the inhibitory effects of hydrocortisone on PMN receptor activity might be responsible, at least in part, for the increased incidence of infection in patients treated with high doses of this or related anti-inflammatory steroids.

Financial support for the Italian Ministry of Public Education is gratefully acknowledged.

March 8, 1979

Note in Proof

Crabtree et al (1979) have just reported that cortisol at 1 μM reduces by approximately 50% the number of Fc receptors on a human granulocytic cell line.

REFERENCES

Crabtree, G. R., Munck, A., Smith, K. A. (1979) Nature (London) 279: 338–339

Ehlenberger, A. G., Nussenzweig, V. (1977) J. Exp. Med. 145: 357–371

Katler, E., Weissmann, G. (1977) Inflammation 2: 295–307

Rinehart, J. J., Balcerzak, S. P., Sagone, A. L., LoBuglio, A. F. (1974) J. Clin. Invest. 54: 1337–1343

Schreiber, A. D., Parsons, J., McDermott, P., Cooper, R. (1975) Ibid. 56: 1189–1197

Tolone, G. (1968) Riv. Ist. Sieroter. Ital. 43: 292–303