New Anti-Inflammatory Proteins Secreted by Human Glucocorticoid-Treated Macrophages

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Glucocorticoids (GCs) exert their anti-inflammatory effects by modulating functions of various cell types including macrophages. We recently found that GCs induce in vitro and in vivo the formation of a novel macrophage subtype defined by the monoclonal antibody RM 3/1, which is associated with the down-regulation of inflammation [1, 2]. Here we report that these macrophages produce factors with anti-inflammatory activity.

Blood monocytes were cultivated for 40 h under serum-free conditions in the absence or presence of the GC pred-nilydene (10^{-7} M). As expected, the GC increased the percentage of RM 3/1+ macrophages to about 80% [1,2]. Control cultures revealed less than 5% RM 3/1+ cells.

The macrophage homogenates and their culture super-natants were tested in the serotonin-induced footpad oedema of the mouse, a model for the early phase of inflammation, and the carrageenan-induced footpad oedema of the rat, a model for the late, eicosanoid-dependent phase of inflammation [3].

Proteins from supernatants of GC-treated macrophages inhibited both inflammatory reactions applied systemically subcutaneous 1 h before elicitation of the oedema. Supernatants from control cells or cell extracts had no effect. This indicates that the inhibitory activity is secreted by macrophages. The activity was found to be dose dependent reaching the same anti-inflammatory potency as a systemically applied GC (0.5 mg/kg dexamethasone, 6h before elicitation). The activity was destroyed by either treatment with heat (1 h, 80°C) or trypsin or proteinase K but not with amy-lase, lipase, chymotrypsin or protease from *Staphylococcus aureus*. This suggests a protein nature for the anti-inflammatory material.

First purification steps, chromatography on DEAE cellulose (pH 7.4) followed by Sephadex G200 or sephacel, and electrophoretic analysis showed that the serotonin oedema and the early phase (80 min) of the carrageenan oedema were strongly inhibited by a protein of about 80 kD and by some peptides of < 10 kD. In contrast, the late phase of the

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carrageenan oedema was only inhibited by proteins of 10-60 kD, presumably the Hpocortins. These GC-inducible proteins, particularly lipocortin-1, have been reported to act on the late, eicosanoid-mediated phase but not on the early phase of inflammation [3,4].

Our results show that GC-induced RM 3/1+ macrophages release proteins inhibiting the early phase of inflammation. This makes them distinct from the Hpocortins [4]. The molecular weights and the GC inducibility also suggest that these proteins are different from other anti-inflammatory proteins such as interleukin-10 [5].

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