REGULATION OF NEUTROPHIL FUNCTIONS BY TUMOR NECROSIS FACTOR-ALPHA

by

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CHAPTER 8 GENERAL DISCUSSION

SUMMARY

Neutrophils are essential for the successful development of an inflammatory reaction, and a defect in any of their inflammatory functions reduces their microbicidal capacity. Since TNF-α is produced by macrophages at the site of infection, its influence on neutrophil functions was studied.

In the first series of experiments, two functions were studied - the generation of superoxide anion, and neutrophil locomotion. rH TNF-α was unable to directly induce the release of superoxide anion from neutrophils, but could enhance the response to the chemotactic peptide *N*-formylmethionylleucylphenylalanine (f Met-Leu-Phe). Three characteristics of priming emerge from this study. Firstly, the response to f Met-Leu-Phe varies between donors, and this affects the subsequent degree of enhancement induced by rH TNF-α. Secondly, the cells must be preexposed to rH TNF-α for an enhanced response to f Met-Leu-Phe to occur, and thirdly, washing the rH TNF-α from the cells prior to stimulating them with f Met-Leu-Phe did not abolish priming. Control experiments were performed to determine the specificity of this response. When cells were stimulated in the presence of superoxide dismutase, priming by rH TNF-α was abolished, indicating that neutrophils specifically increase their production of superoxide anion in response to priming by rH TNF-α. The possibility of contamination by LPS was discounted by incubating the cells in the presence of boiled and untreated rH TNF-α. It was found that the primed response, but not the response to f Met-Leu-Phe was abolished by boiling rH TNF-α. To define whether priming was confined to the f Met-Leu-Phe response, the effect of rH TNF-α was tested on two other activators of neutrophil function. Preincubation with rH TNF-α enhanced the production of superoxide anion in response to zymosan activated serum (crude C5a), but not to phorbol myristate acetate.

Another aspect of neutrophil function required for the development of an inflammatory response is the chemotactic migration from the blood to the site of infection. Although rH TNF-α was not itself chemotactic for neutrophils, preincubation with this cytokine inhibited the chemotaxis of neutrophils towards a source of f Met-Leu-Phe. The inhibition of chemotaxis was dependent on both the concentration of rH TNF-α used, and the time of preincubation. In addition, the inhibition of chemotaxis was not confined to f Met-Leu-Phe, because rH TNF-α was also shown to inhibit the migration towards a gradient of zymosan activated serum.

The second series of experiments were designed to further define the influence of rH TNFα on neutrophil superoxide generation and chemotaxis, and attempt to define the mechanism by which rH TNF-α enhanced superoxide production but inhibited chemotaxis. rH TNF-α was found to enhance superoxide production in a concentration-dependent manner. In parallel experiments, a titration of f Met-Leu-Phe in the presence or absence of rH TNF-α revealed that neutrophils generate more superoxide anion at concentrations of f Met-Leu-Phe from 10^{-6} to 10^{-8}M. As with the inhibition of chemotaxis, enhancement of superoxide generation by rH TNF-α occurred in a time -dependent manner, and exhibited similar kinetics when compared to the chemotactic response. Unstimulated neutrophils possess f Met-Leu-Phe receptors with high and low affinities, which are thought to be responsible for the chemotactic and superoxide reponses respectively. The regulation of f Met-Leu-Phe receptor expression on human neutrophils was therefore studied. Upon incubation with rH TNF-α, the high affinity f Met-Leu-Phe receptor was lost, and a single lower affinity receptor population was expressed. Although the affinity of the receptors was altered, the total number of f Met-Leu-Phe receptors remained unchanged. The changes in receptor expression were consistent with the increase in superoxide production and the decrease in chemotactic responsiveness.

To investigate whether the regulation of superoxide generation, chemotaxis, and f Met-Leu-Phe receptor expression was unique to rH TNF-α, the effect of recombinant human

granulocyte-macrophage colony-stimulating factor (rH GM-CSF) on neutrophil responses and receptor expression was studied. As with rH TNF-α, rH GM-CSF enhanced the respiratory burst in a concentration- and time-dependent manner. Preincubation with rH GM-CSF also inhibited chemotactic migration in a time-dependent manner, which paralleled that shown for superoxide anion generation. F Met-Leu-Phe receptor affinity but not number, was also altered in a manner similar to that shown for rH TNF-α. Interestingly, the time course of change in receptor expression was similar to the time courses for superoxide generation and chemotaxis. These observations imply that the regulation of f Met-Leu-Phe receptor affinity may contribute to the regulation of neutrophil functions by rH TNF-α.

The other microbicidal mechanism of neutrophils is the release of lysosomal enzymes into phagosomes and the extracellular environment. To further define the regulation of neutrophil function by rH TNF-α, its influence on neutrophil degranulation was studied. In contrast to superoxide anion production, rH TNF-α directly stimulated neutrophil degranulation. Preincubation with rH TNF-α also enhanced the response to f Met-Leu-Phe, and although the effect was additive, it varied amongst donors as for superoxide generation. Preincubation with rH TNF-α influenced neutrophil degranulation in response to f Met-Leu-Phe in a concentration-dependent manner, and exhibited an additive effect at all f Met-Leu-Phe concentrations tested. The cell surface expression of the granule associated receptor for C3bi (CR3) was also studied. As with degranulation, rH TNF-α directly stimulated the expression of this molecule, but the effect was never as potent as that observed for f Met-Leu-Phe.

Neutrophil degranulation is dependent on extensive actin rearrangements within the neutrophil cytoskeleton, therefore the effect of rH TNF-α on actin polymerisation was studied. Incubation of neutrophils with rH TNF-α stimulated the rapid polymerisation of actin which peaked within 10 seconds and depolymerised within two minutes. Although rH TNF-α-induced polymerisation was kinetically similar to f Met-Leu-Phe-induced actin

polymerisation, both the magnitude of the response and the depolymerisation time were always less. The pattern of actin polymerisation was similar between rH TNF-α and f Met-Leu-Phe, and a titration of rH TNF-α revealed that this effect could only be detected at 1000 and 100 u/ml, which is higher than that required for other functions. In addition to a direct effect on actin polymerisation, preincubation with rh TNF-α also enhanced f Met-Leu-Phe-induced actin polymerisation in a concentration-dependent manner.

The neutrophil functions that rH TNF-α directly affects, i.e. cell surface receptor expression, degranulation and actin polymerisation, all require some alteration in the lipid composition of the cells. In addition to this, certain neutrophil lipids act as second messengers in the signal transduction pathways. Therefore, to determine whether rH TNFα could influence lipid metabolism, the release of arachidonic acid from neutrophils was studied. Incubation with rH TNF-α induced the release of arachidonic acid from neutrophils. Interestingly, this effect was equipotent with that observed for f Met-Leu-Phe, and appears to be confined to the cytokines that enhance superoxide production in response to f Met-Leu-Phe. As with the other effects observed, the stimulation of arachidonic acid release from neutrophils was both time- and concentration-dependent. Of particular note, the time course of rH TNF-α-induced arachidonic acid release appears to be temporally related to that observed for the enhancement of superoxide anion generation and inhibition of chemotaxis. In addition, although rH TNF-α induces the release of arachidonic acid from neutrophils, it does not appear to stimulate its metabolism. These observations suggest a possible mechanism of priming of peripheral blood neutrophil functions by rH TNF-α.