MORPHOLOGICAL RESPONSES OF NEUTROPHILS
IN SUSPENSION
TO PLASMA COMPONENTS AND CHEMOTACTIC FACTORS

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SUMMARY

Neutrophil leukocytes develop polarised cytoplasmic extensions as a prerequisite for their emigration into inflamed tissues. This shape-change has been extensively studied in cell suspensions as a response of neutrophils to chemotactic factors such as the synthetic bacterial peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP). However, neutrophils also polarise when suspended in fresh heparinised plasma and the details of this response have not been previously characterised.

This study examined the time course and degree of neutrophil polarisation in plasma and compared this response with those induced by FMLP, purified plasma proteins (particularly immunoglobulin type G) and chemotactic inflammatory mediators (the complement derived fragment C5a, interleukin-8, leukotriene B4 and platelet activating factor). In addition, the possible roles of extracellular divalent cations (Ca^{2+} and Mg^{2+}), intracellular Ca^{2+} ions and actin microfilament distribution during responses to each stimulus were examined.

Neutrophils were isolated from human peripheral blood by a one-step Hypaque-Ficoll method and resuspended in Hank's balanced salt solution buffered with 20 mM Heps (HBSS-Heps) containing test agents, at 37 °C. Polarisation was assessed by microscopic examination (Nomarski optics) and classification of glutaraldehyde (2.5% v/v in phosphate-buffered saline)-fixed cells into five morphological subtypes: spherical (unstimulated); type 1 cells, characterised by non-polarised extensions; type 2 cells, characterised by polarised extensions and round body; type 3 cells, characterised by polarised extensions and an oval body; and type 4 cells (fully polarised), characterised by polarised extensions including a tubular (uro-pod). Optimal responses to each stimulus were defined as those exhibiting the greatest proportion of type 4 cells. Computerised morphometry and fluorescence activated cell sorter (FACS) analysis were examined as methods for assessing
polarisation, but neither technique was satisfactory since morphometry did not consistently distinguish between non-polarised (type 1 cells) and polarised cells (types 2 and 3) and FACS analysis only detected changes in cell size.

Standard preparations of heparinised (12.5 I.U./ml) plasma (10%, 50% and 99% v/v) induced immediate (within 30 seconds) polarisation responses which were apparent throughout the 60 minutes incubation, but cells rarely developed a type 4 morphology. In contrast, plasma anticoagulated with ethylene-bis-(oxyethyl)-enentriol-tetra-acetic acid (EGTA, 5 mM) or low concentrations of heparin (2.5 I.U./ml), standard plasma pre-treated for 5 minutes with soy bean trypsin inhibitor (0.25 mM) or additional Mg^2+ ions (0.5 to 5 mM), and fresh serum, induced formation of many type 4 cells. Plasma anticoagulated with either ethylene-diamine-tetra-acetic acid (EDTA, 5 mM) or disodium hydrogen citrate (4 mg/ml) induced little or no change in cell shape.

Responses to standard preparations of heparinised plasma were reduced in the presence of chelating agents of extracellular cations (EDTA, EGTA, or disodium hydrogen citrate; 5 mM). These inhibitory effects of chelating agents did not occur if compensatory concentrations of either additional Ca^{2+} or Mg^{2+} ions (5 mM) were present.

Commercial preparations of IgG (0.009% and 0.0005% w/v) containing aggregates induced neutrophil polarisation, but this effect was delayed until 5 minutes. Reducing the proportion of aggregated IgG by ultra-centrifugation further delayed or abolished responses. Partial removal (99%) of the type II receptor for IgG on neutrophils did not affect the response of the cells to this protein. Fc and F(ab) fragments of IgG prepared by papain digestion did not induce polarisation.

Heat aggregated preparations of IgG (0.1%, 0.01% and 0.001% w/v) induced neutrophil polarisation with high proportions of type 4 cells at higher concentrations, but these responses were also generally delayed until 5 minutes. In contrast, supernatants from cells suspended in 0.01% heat aggregated IgG for 6 minutes induced an immediate (within 30 seconds) response in fresh cells. Responses to 0.1% and
0.01% (but not 0.001%) heat aggregated IgG were markedly reduced in the presence of EDTA, but all responses were unaffected by EGTA.

Optimal responses to each chemotactic factor were observed at 10 nM for FMLP and leukotriene B4, 1 nM for C5a, 12.5 nM for interleukin-8, and 40 to 400 nM for platelet activating factor. These responses were rapid in onset (within 30 seconds), sustained for at least 60 minutes and were characterised by moderate to high numbers of type 4 cells. Furthermore, in contrast to the responses in plasma and IgG, responses in each chemotactic factor were generally enhanced in the presence of chelating agents of extracellular divalent cations.

Responses to plasma, IgG and chemotactic factors were generally abolished by pre-treating cells with 8-(diethylamino)octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8; 5 x 10-4 M for 10 minutes), an inhibitor of the release of intracellular Ca2+ ions. Furthermore, cells treated with plasma, IgG or chemotactic factors consistently displayed abundant F-actin within their cytoplasmic extensions when stained with the fluorescent F-actin probe rhodamine phalloidin.

The present studies demonstrate significant differences between the polarisation responses of neutrophils in suspension to heparinised plasma, IgG and chemotactic factors. This implies that motile responses of neutrophils in inflamed tissues may vary according to the cause and mediation of the inflammatory response. In addition, these findings suggest that plasma derived factors other than established chemotactic stimuli may regulate the emigration of neutrophils in vivo.