EFFECT OF FACTOR H ON THE FUNCTION OF HUMAN NEUTROPHIL GRANULOCYTES

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Background: Factor H (FH) is a major inhibitor of the alternative pathway of complement activation in plasma and on host surfaces. In addition, FH has been shown to bind to neutrophil granulocytes presumably via complement receptor type 3 (CR3; CD11b/CD18) and mediate adhesion. The cellular roles of FH are, however, poorly understood. Because neutrophils are important innate immune cells in inflammatory processes and the host defence against pathogens, we aimed at studying the effects of FH on various neutrophil functions.
Methods: Human neutrophil granulocytes were isolated from peripheral blood of healthy individuals. Neutrophil spreading was monitored by laser-scanning confocal microscopy using fluorescent F-actin probe to measure the contact zone area. Co-localization between FH and CD11b was quantified by calculating Pearson's correlation coefficients from at least 100 cells. Cell migration assays were performed in serum-free RPMI-1640 medium using transwell plates with 3-µm pore polycarbonate membranes. After induction of neutrophil extracellular traps (NETs), DNA was stained with Sytox Orange. The relative fluorescence intensity of adhered neutrophils (Cell Tracker Green labelled), the extracellular DNA (NET formation) and ROS production (dihydrorhodamine 1,2,3 oxidation) were measured in 96-well black transparent-bottom plates using a fluorescence reader. IL-8 was measured from cell culture supernatants by ELISA. The cell activating capacity of FH was monitored by Ca^{2+} measurement on flow cytometry and by anti-phospho tyrosine Western blot techniques.
Results: FH showed co-localization with CD11b. Cell surface-bound FH retained its cofactor activity and enhanced C3b degradation. FH did not induce Ca^{2+} signal in the cells, but altered the tyrosine phosphorylation pattern. FH supported neutrophil migration and spreading. In addition, immobilized FH enhanced IL-8 release from neutrophils. FH alone did not trigger the cells to produce NETs. In contrast, NET formation induced by PMA and by fibronectin plus beta-glucan were inhibited by FH. This inhibition was specific since the major CR3 ligand iC3b did not elicit such an inhibitory effect. Since we measured similar cell numbers in each sample, it can be excluded that the differences were due to altered adhesion properties. Moreover, in parallel with NET formation, FH also inhibited ROS production.
Conclusions: These data show that FH has multiple regulatory roles on neutrophil functions. While it might support the recruitment of neutrophils, FH – depending on the stimulus context – could also exert anti-inflammatory effects and influence local inflammatory and antimicrobial reactions as well as tissue damage by modulating NET formation.