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Activation of human neutrophils by C3a and C5A

Comparison of the effects on shape changes, chemotaxis, secretion, and respiratory burst

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Abstract

The effects of anaphylatoxin C3a on human neutrophils were studied in comparison with C5a. Both peptides induced a transient shape change response and a respiratory burst. In both cases C3a was 50- to 100-times less potent than C5a. A marked chemotactic response with bimodal concentration dependence was obtained with C5a, but no neutrophil chemotaxis was observed with C3a. Repeated stimulation led to homologous desensitization of shape changes and respiratory burst but no cross-desensitization, indicating that the two anaphylatoxins act through separate receptors. The lack of chemotactic activity suggests that C3a is not involved in neutrophil recruitment into infected or inflamed tissues.

Key words: Complement; Inflammation; Shape change; Exocytosis; Respiratory burst; Desensitization

1. Introduction

The anaphylatoxins C3a and C5a are generated upon complement activation, and are known for their proinflammatory effects [1–3]. C5a is generally recognized as a mediator of inflammation with potent attractant and activating effects on neutrophil, eosinophil and basophil leukocytes [4–7], while the role of C3a is less defined. It has been shown that C3a binds to neutrophils [8,9], eosinophils and basophils [10]. It induces neutrophil aggregation [11,12] and morphological changes [13], but little or no enzyme release [9,14]. In neutrophils [15] and differentiated U937 cells [8,9], C3a increases the cytosolic free Ca^{2+} concentration by a G protein-dependent signal transduction pathway different from the one involved in C5a-mediated neutrophil stimulation.

We studied the responses of human neutrophils to C3a with special attention to functions that are related to antimicrobial defence, i.e. the induction of motile behavior, migration, and the respiratory burst. The data show that C3a activates the motor apparatus of the cells (as already implied in [13]) and elicits a characteristic, albeit weak, respiratory burst, but does not induce chemotaxis in vitro. It thus appears that C3a plays only a minor role in neutrophil recruitment.

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Abbreviations: C3a, serum complement fragment 3a; C5a, serum complement fragment 5a (anaphylatoxins).

2. Materials and methods

2.1. Materials

Stock solutions of homogenous C3a and C5a prepared from human serum [5] were kindly provided by Dr. C.A. Dahinden, Department of Clinical Immunology, University of Bern, Switzerland; they were further diluted with test buffer (below) containing 0.25% fatty acid-free BSA (Boehringer-Mannheim, Rotkreuz, Switzerland). All other chemicals were obtained as described in [16].

2.2. Cell preparations

For the chemotaxis assays, neutrophils were isolated by dextran sedimentation of freshly drawn blood from healthy volunteers according to [17]. All other assays were performed using neutrophils prepared from donor blood buffy coats [16]. The purified cells were suspended in test buffer containing (mM) 130 NaCl, 5.0 $NaHCO_3$, 4.6 KCl, 5 glucose, 0.05 $CaCl_2$, and 20 HEPES, adjusted to pH 7.4 with NaOH, and kept above ice at ca. 10°C.

2.3. Functional assays

Shape change was assayed by measuring the agonist-induced changes in light transmission and 90° scattering of stirred neutrophil suspensions (8×10^5 cells/ml) at 37°C [18]. Extinction was calculated from the transmission by means of Beer's law.

Chemotaxis was assessed in multi-well chemotaxis chambers (Neuro Probe, Cabin John, MD) by the method described in [19].

Exocytosis of β-glucuronidase was determined in neutrophils pretreated with cytochalasin B according to the procedure of Dewald and Baggiolini [20].

The respiratory burst was followed using a previously described real-time chemiluminescence assay for H_2O_2 [21].

3. Results

3.1. Shape change

Transient changes in extinction and 90° light scattering were observed after stimulation of suspended neutrophils with either C3a or C5a (Fig. 1). The amplitude of the peak response was similar for both anaphylatox-

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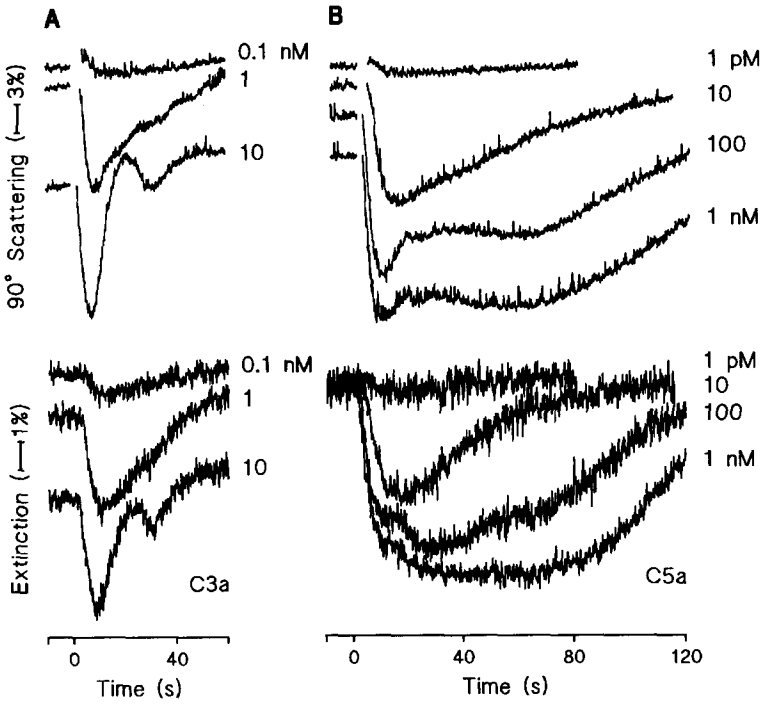

Fig. 1. Shape change responses of human neutrophils. Percent change from resting level in 90° light scattering (top) and extinction (bottom) of cells stimulated with C3a (A) or C5a (B) at time 0. The tracings are representative of 3 experiments performed with cells from different donors.

ins, but the progress curves were dramatically different and the duration of the C3a response was significantly shorter. A plot relating maximum extinction change to stimulus concentration shows that C5a was about 50-fold more potent than C3a as a stimulus of neutrophil shape change (EC_{50} = 0.007 and 0.37 nM, respecively; Fig. 2).

3.2. *Other functional responses*

The ability of C3a to induce chemotaxis, secretion and a respiratory burst was tested. C3a was not chemotactic in vitro over a concentration range of 6 orders of magnitude (0.01–1,000 nM) and was virtually inactive as a stimulus of exocytosis: only borderline amounts of β-glucuronidase were released from cytochalasin B-treated cells up to the highest concentration tested (100 nM). By contrast, C5a was a powerful inducer of both chemotaxis and exocytosis. Significant chemotaxis was detected with as little as 50 pM C5a, rising to a maximum at 1 nM and declining thereafter (Fig. 3). At 10 nM, C5a was four times more potent than C3a in stimulating release of β-glucuronidase from cytochalasin B-treated cells. Both anaphylatoxins induced a rapid, transient respiratory burst of roughly the same duration (Fig. 4). However, the total yield of H_2O_2 was more than 100-fold higher upon stimulation with C5a.

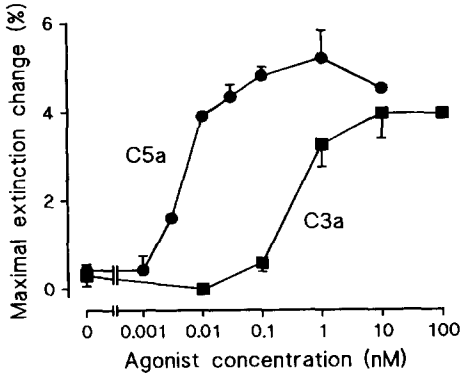

Fig. 2. Concentration dependence of neutrophil shape change responses induced by C3a and C5a. Mean values ± S.D. of maximal extinction changes 10–15 s after stimulation obtained in 3–7 experiments performed with cells from different donors.

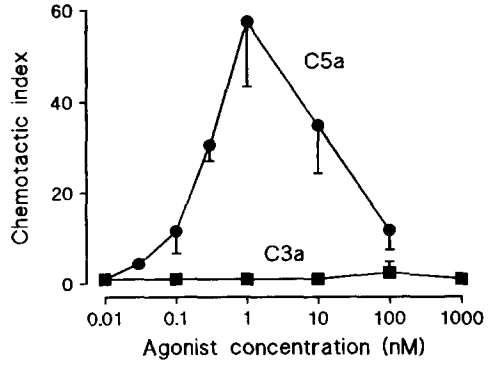

Fig. 3. Chemotactic migration of human neutrophils after stimulation with C3a or C5a. Mean values ± S.D. of the chemotactic index from 3 experiments performed with cells from different donors.

3.3. Receptor desensitization

Repeated stimulation of neutrophils with C3a led to a loss of responsiveness in respiratory burst (Fig. 4) and shape change response (Fig. 5). Cells that were fully desensitized to C3a, nonetheless, responded normally to C5a (Figs. 4 and 5). Prestimulation with C3a did not influence the subsequent shape change or respiratory burst response to C5a, and prestimulation with C5a did not influence responses to C3a (Fig. 5). These data strongly suggest that the two anaphylatoxins exert their effects via different receptors.

4. Discussion

Neutrophils responded to the anaphylatoxic peptides C3a and C5a with a marked shape change that was similar in amplitude, but shorter for C3a. C3a also provoked a respiratory burst (with a yield of H_2O_2 that was less than 1/100th of that observed with C5a), but was virtually inactive as a stimulus of exocytosis, and did not induce chemotaxis. Desensitization experiments showed

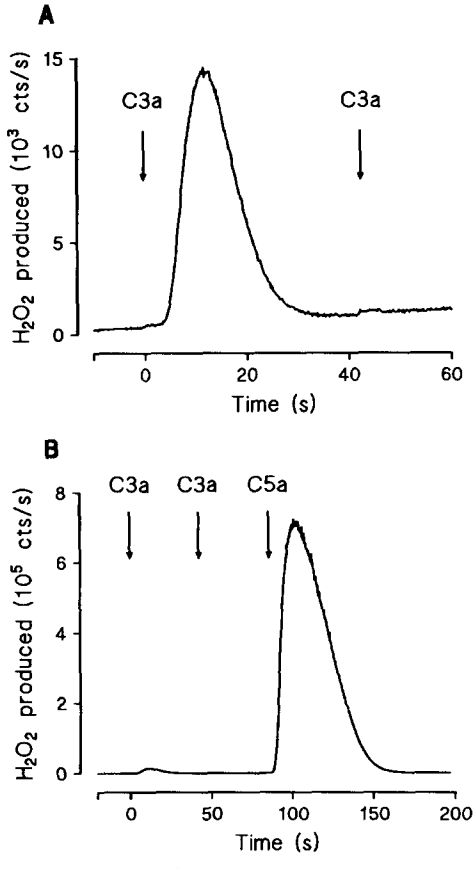

Fig. 4. Respiratory burst of human neutrophils after stimulation with C3a or C5a. The responses to 40 nM C3a or 10 nM C5a, added to the cells as indicated by arrows, are shown. Panel A shows the first 60 s of the tracing in panel B (note the difference in vertical scale between panels A and B). This result is representative of 3 experiments performed with cells from different donors.

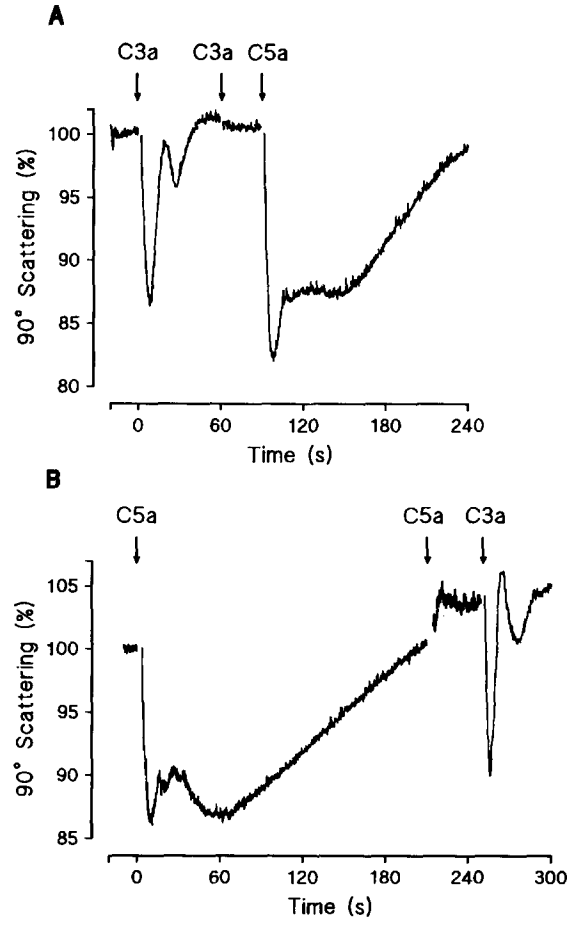

Fig. 5. Cross-desensitization experiments after homologous desensitization with C3a (A) or C5a (B). 90° scattering changes of neutrophils stimulated repeatedly with C3a (10 nM) or C5a (0.1 nM in A, 30 nM in B; arrows). The tracings are representative for 3–4 experiments performed with cells from different donors.

that C3a and C5a did not influence each others responses, indicating, in agreement with previous reports [9,12], that the two anaphylatoxins act via separate receptors.

The unequal time-course of the shape change transients may suggest that C3a and C5a have different effects on the cytoskeleton. Alternatively, Yuli and Snyderman pointed out that the 'slow' scattering response could reflect secretion [22]. Thus, the short duration of C3a-mediated scattering changes may reflect the inability of C3a to induce exocytosis. Although the maximal amplitudes of the shape change responses were similar, C3a was about 50-times less potent than C5a. The difference in potency could be partly due to the numbers and affinities of the respective receptors (neutrophils have 30,000–40,000 C3a [8,9] and 100,000–200,000 C5a receptors [23,24] with mean K_d values of 2–30 and 1–2 nM, respectively). Our data agree with similar potency differences described in earlier reports for leukotriene B_4 formation [25] and cell aggregation [11,12] induced by these two anaphylatoxins in neutrophils.

It is interesting that C3a, despite its pronounced effect on shape, was completely inactive as a chemoattractant, since shape change is considered a correlate of migration [26]. Other events, however, are required for chemotaxis, and C3a might be inactive in the expression of adhesion receptors which are necessary for neutrophil migration on a substrate.

Together with recently reported data from other laboratories, the present results demonstrate that C3a activates human neutrophils, although its potency is considerably lower than that of C5a. The major difference between these anaphylatoxins is in chemotaxis and secretion, which are induced by C5a, but not by C3a. The inability to induce chemotaxis suggests that C3a plays a lesser role than other neutrophil-activating agonists and chemotactic cytokines in neutrophil-dependent antimicrobial defence.

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