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Enhancement of Macrophage Phagocytosis by Complement Component C5a

(macrophage/phagocytosis/C5a)

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To investigate the modulatory function of complement component C5a in macrophages, the effects of C5a on macrophage phagocytosis were examined by means of fluorescent polystyrene particles and flow cytometry. We found that C5a enhanced the phagocytosis of latex beads in mouse peritoneal macrophages. The results suggest that the anaphylatoxin C5a can modulate macrophage functions and may play an important role in specific immune responses as well as in a non-specific immune mechanism.

Complement component C5a is an anaphylatoxin with chemotactic activity for neutrophils and monocytes [11]. In addition to recruiting neutrophils and monocytes to the sites of inflammation, C5a also stimulates other important biological reactions in these leukocytes such as degranulation, activation of oxidative metabolism, and increased expression of CR1 and CR3 [11]. These alterations in leukocytes are key to their participation in both inflammatory and immunological reactions. Because the effects of C5a on macrophage phagocytosis is not known, we have studied the effects of C5a on phagocytosis in mouse peritoneal macrophages by using flow cytometry.

Primary macrophage cultures were prepared as described previously [5] by injecting 4ml thioglycollate medium into the peritoneum of BALB/cA Jcl mice (Nihon Clea, Tokyo). The cells were cultured in RPMI 1640 solution containing 10% fetal bovine serum, 100μg/ml streptomycin and 100 units/ml penicillin G in a CO_2 incubator at 37℃. The phagocytic assay was conducted within a week after cells were plated onto plastic culture dishes (35mm).

Phagocytosis was analyzed by flow cytometry (FACStar, Becton Dickinson Immunocytometry System, Mountain View, CA, USA) as described previously [5]. Macrophages were incubated in the presence or the absence of C5a with fluorescent particles at a density of 10^7 particles/dish for 30-60 min in normal external solution, which consisted of 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.5. Adhered cells were detached by trypsinization (0.25%) and subjected to flow cytometric analysis. The percentage of phagocytic cells (PP) was defined as the percentage of macrophages that ingested one or more particles. The phagocytic index (PI) was defined as the average number of particles ingested per macrophage and was calculated by dividing the total number of ingested beads by the total number of macrophages.

Human recombinant C5a was purchased from Sigma Chemical Co., St. Louis, MO, USA; fluorescent latex particles (no. 18338, 2μm diameter) were from Polysciences, Warrington, PA, USA; thioglycollate, PBS, Hanks and RPMI 1640 medium from Nissui Pharmac., Tokyo. Other reagents were purchased from Nacalai Tesque, Kyoto.

The figure shows contour plots of phagocytic cells which are based on forward scattering (FSC) versus side scatter (SSC) and FSC versus relative fluorescence intensity (FL), as shown in a1, a2, b1 and b2 of the figure. The left clusters in a2 and b2 represent the cell population which does not ingest any bead and the right ones represent the bead-ingested

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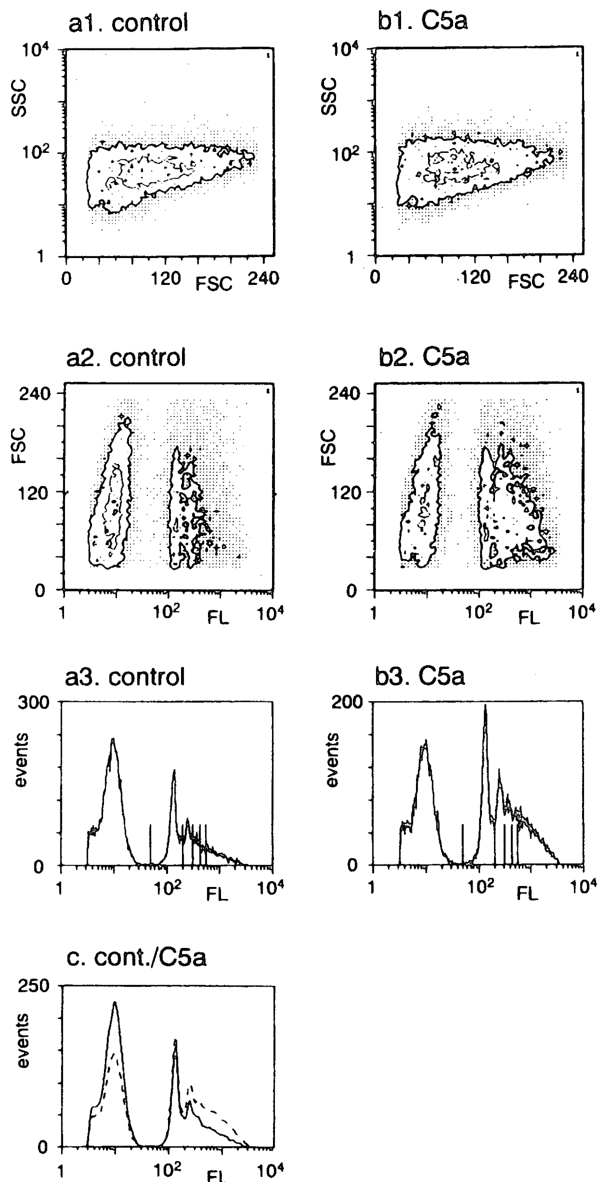

Effects of C5a on flow cytometric profiles. (a1) A contour plot of cells based on forward (FSC) and side (SSC) scatter. (a2) A control contour plot of fluorescence (FL) versus FSC in the upper plot. Each dot shows the area at a cell density of 1 to 4 cells/unit square. Contour lines indicate an increase in cell density per 15 cells. (a3) Histogram of cells based on FL intensities in the upper plots. Non-ingested, 1 bead-ingested, 2 beads-ingested cell populations are indicated from the first, second and third peaks from the left, respectively. b1, b2 and b3 are contour plots and a histogram; FSC vs. SSC, FL vs. FSC and cell number vs. FL in the presence of C5a (10^{-8} M), respectively. (c) Superimposed graphs consisting of control and C5a-enhanced phagocytosis from a3 and b3.

macrophages. The distributions of cell numbers versus FL intensity are shown in a3 and b3 of the figure. The right plots and graph in b1, b2 and b3 are contour plots and a histogram in the presence of C 5a. Comparing a1 and a2 with b1 and b2, the areas and contour height of the non-ingested cell populations were decreased and those of the bead-ingested cell populations were increased in the presence of C5a. The same result is shown in the superimposed histogram of Fig. c. These data indicate that C5a enhanced phagocytosis of latex beads in mouse peritoneal macrophages.

During the activation of complement, a variety of cleavage fragments are produced which display potent inflammatory, immunomodulatory and immunological properties. One of these fragments is C5a, a 74-residue glycopeptide produced by cleavage of the amino terminal end of the C5α chain. C5a is well known as an anaphylatoxin and chemotactic factor for macrophages [11]. Many other activities of C5a are also demonstrated even in monocytes and macrophages. C5a induces macrophages and a macrophage cell line to secrete interleukin-1 [3]. In addition, C5a enhances interleukin-1 and tumor necrosis factor release by lipopolysaccharide-stimulated monocytes and macrophages [1]. Interleukin-8 synthesis and its release from monocytes is also induced by C5a [2]. Furthermore, C5a induces an increase in intracellular Ca^{2+} by the release of intracellular Ca^{2+} stores and influx of extracellular Ca^{2+} in U937 monocytic cells [8]. We have found that C5a activated K^+ outward current in mouse peritoneal macrophages [4]. In brain macrophages, microglia, C5a also stimulates motility [9], activates K^+ outward current [6] and induces intracellular Ca^{2+} elevation mediated by pertussis toxin-sensitive G-proteins [7]. These data indicate that C5a can modulate many physiological functions in macrophages.

Though an enhancement of phagocytosis in human neutrophils by C5a has been demonstrated [10], the effects of C5a on macrophage phagocytosis have not been investigated. The present study directly demonstrated that C5a could enhance the phagocytic activity of mouse peritoneal macrophages. Concerning phagocytosis, it is known that in monocytes and neutrophils C5a induces increases in the expression of the C3b receptor (CR1) and the iC3b receptor (CR3) [12] which are responsible for binding opsonized particles, phagocytosis and the immune adherence reaction by circulating and tissue-fixed mononuclear phagocytes. So, the phagocytic enhancement observed in the present study may

involve CR1 and/or CR3 receptors of macrophages.
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