Release of Arachidonic Acid by Stimulation of Opsonic Receptors in Human Monocytes

THE FcγR AND THE COMPLEMENT RECEPTOR 3 PATHWAYS*

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The role of the opsonic receptors FcγR and CR3 on the release of arachidonic acid (AA) by human monocytes was studied using IgG-ovalbumin (OVA) equivalence immune complexes (IC), anti-OVA IgG bound to OVA-coupled latex beads, and C3bi-bound IC. Release of AA was produced by IC and latex-ova beads bound to IgG, whereas binding of C3bi to IC inhibited the ability of IC to release AA. In contrast, coating of zymosan particles with C3bi enhanced AA release as compared with that produced by non-coated particles. Masking of C3bi on C3bi-bound IC by incubation with anti-C3 IgG resulted in the recovery of their ability to release AA, thereby suggesting that binding of C3bi by IC reduces their flogogen effects, whereas opsonization of microbial walls by binding complement may enhance their proinflammatory potential. The binding/uptake of opsonized zymosan particles was inhibited by anti-CR3 Ab and C3bi-bound IC, but not by β-glucan, mannan, and anti-Toll-like receptor 2 Ab. These findings show that cooperative engagement of CR3 on both the lectin-like site involved in β-glucan binding and the I-domain involved in C3bi binding, as it can be observed in the innate immune response, produces AA release, whereas the unique interaction of C3bi-bound IC with the I-domain of CR3, as it may occur in the adaptive immune response, diverts the IC lattice from a productive interaction with FcγR linked to AA release.

The purported sequence of events following antigen exposure in animals sensitized with IgG Ab† is the formation of immune complexes (IC) as soon as antigens encounter their cognate Ab. This is accompanied with the covalent binding of C3 to the Ag-Ab lattice when IC formation takes place in biological fluids containing complement, due to the high reactivity of the internal thiosteerger of C3, which becomes exposed to the solvent upon proteolytic activation of C3 (1). IC containing covalently bound C3b have the capacity to interact with receptors for the Fc portion of the IgG molecule (FcγR) and with CR3 (complement receptor type 3, Mac-1, αMβ2 integrin, CD18/CD11b). The CR3 integrin is the main receptor for C3bi, the major product of IgG-bound C3b as a result of the action of the complement regulators.

The study of several models of immune-mediated tissue injury in mice with a targeted disruption of the γ-chain of FcγR has made it possible to discriminate between IC deposition and the inflammatory response produced by IC, thereby depicting a model of IC-mediated injury in which FcγR-mediated events rather than the complement system are critical to the tissue damage (2, 3). However, the existence of a complex regulatory interplay between complement and the FcγR route is supported by a number of observations: (i) the solubilization and clearance of IC by complement (4), (ii) the inhibition of the interaction of IC with FcγR in lymphocytes produced by the covalent binding of C3bi to Fc (5), and (iii) the hypersensitivity to renal injury by IC elicited by low plasma levels of C3 (6). Furthermore, clinical studies have shown an increased incidence of immune-mediated diseases in patients with reduced levels of complement factors (7).

Studies of several models of immune-mediated inflammation have disclosed a role for lipid mediators in the mediation of the early extravasation of protein-rich plasma (8) and in the late inflammatory response (9), whereas the recruitment of leukocytes is mainly explained by the production of chemokines (10). These responses have been related to the cross-linking of FcγR receptors; however, most studies have been conducted in mice, which express FcγR subtypes different from those present in human cells (13, 14). For instance, murine leukocytes express the inhibitory FcγRII receptor (the murine homologue of human FcγRIIB), whereas human monocytes express FcγRIIIA and FcγRIII is only detected in about 10% of human blood monocytes (15).

In this study we have addressed the effects of both native IC and IC covalently bound to C3bi (C3bi-IC) on the release of arachidonic acid (AA) by human mononuclear cells, and compared these effects with those elicited by C3bi attached to other structures, for instance zymosan particles and F(ab′)2-coated latex beads. Our data show triggering of the AA cascade by FcγR cross-linking in monocytes and monocyte-derived macrophages. However, binding of C3bi to IC diminishes their capacity to release AA, whereas binding of C3bi to zymosan particles has the opposite effect. These data show the existence of a diversity of signaling processes associated to opsonic receptors, which allow coupling of phagocytosis to proinflammatory events under conditions strongly dependent on the nature of the engulfed particle and the presence of associated C3bi molecules.

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The abbreviations used are: Ab, antibody; AA, arachidonic acid; C3, the third component of the complement system; C3bi-IC, immune complexes bound to C3bi; cPLA2, cytosolic phospholipase A2; CR3, complement receptor 3; FcγR, receptor for the Fc portion of IgG; IC, immune complexes; NHS, normal human serum; OVA, ovalbumin; TLR, Toll-like receptor; PBS, phosphate-buffered saline; MAP, mitogen-activated protein.
EXPERIMENTAL PROCEDURES

Reagents—Anti-human CD11b/Mac-1 IgG,

mAb was a gift from Dr. Fernando Vivanco, Fundación Díaz, Madrid, Spain. The IgG fraction of this antisera was purified by FPLC using a protein G-Sepharose column (Amersham Biosciences). Zymosan particles conjugated with Alexa Fluor® 488 were from Molecular Probes Inc. (Eugene, OR). Goat anti-human C3 Ab, zymosan, soluble β-glucan from seaweed (laminarin, ~8 kDa), soluble mannan from Saccharomyces cerevisiae and carbohydrate-modified latex beads were from Sigma Chemical Co. Rabbit polyclonal Ab against Toll-like receptor 2 (TLR-2) and TLR-4 were a kind gift from Drs. Keith Ray and Ginette Squires —

C3. Moreover, this procedure mimics the solubilization of IC deposited by the lack of significant appearance of free antigen in the supernatant buffer. Binding of C3 to IC under the aforementioned conditions does not produce dissociation of antigen-antibody complexes as determined by the absence of appearance of antigen in the supernatant (16). Moreover, this procedure mimics the solubilization of IC deposited in tissues and is characterized by an efficient incorporation of C3b molecules onto the Ab (17). The formation of ester bonds between C3b and IgG γ-chain was demonstrated by the presence of high molecular mass protein adducts sensitive to hydroxylamine treatment (18). The coupling of C3b to C3 was further confirmed by immunoblotting using anti-C3 polyclonal Ab. Zymosan was opsonized with NHS for 15 min at 37°C, followed by extensive washing with PBS.

Preparation of IgG-coated Latex Beads—OVA was coupled to 1-μm carboxylate-modified latex beads by incubation with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, using a two-step procedure to improve OVA incorporation. Coating of the OVA-coupled beads with IgG was carried out by incubation with anti-ova OVA IgG purified by protein G chromatography. Anti-OVA F(ab′)2 was obtained by pepsin digestion of purified IgG from rabbit anti-OVA antiserum, followed by protein A chromatography to remove both undigested IgG and Fc fragments. Coupling of C3b to latex beads was carried out on beads coated with both anti-OVA IgG and F(ab′)2, according to the protocol described for IC. C3b coating to the beads was monitored by SDS-PAGE under reducing conditions.

Cell Culture, Metabolic Labeling, and Assay of [3H]AA Release—THP-1 cells were cultured in RPMI 1640 medium supplemented with 2 mm glutamine and 10% heat-inactivated fetal bovine serum. Human monocytes were isolated from peripheral blood of healthy volunteer donors by centrifugation onto Ficoll cushions and adherence to plastic dishes for 2 h. At the end of this period, non-adhered cells were removed by extensive washing. Radioactive labeling with [3H]AA was performed by overnight incubation of cells in the absence of serum and the presence of 0.25 μCi/ml [3H]AA in 0.25% essentially fatty acid-free bovine serum albumin. After labeling, cells were washed with medium and allowed to equilibrate at 37°C in medium containing 1% bovine serum albumin, before the addition of agonists or vehicle. The release of [3H]AA into the culture medium was measured by scintillation counting. Contamination of platelets under these conditions of cell isolation and labeling was less than 2 platelets per monocyte. Human platelets were obtained by centrifugation of platelet-rich plasma and pelleting, prior to labeling with [3H]AA. Differentiation of monocytes into macrophages was conducted by culture of adherent monocytes in the presence of autologous serum for 2–3 weeks in Primaria 6-well dishes (Falcon Plastics Inc.), in the absence of exogenous cytokine cocktails.

Immuno blotting of cPLA2—Protein extracts were collected from culture dishes in ice-cold extraction buffer containing 50 mM Hepes, 1 mM EDTA, 10% glycerol, 10 μg/ml leupeptin, and 1 μM phenylmethylsulfonyl fluoride, pH 7.4. The amount of protein in each cell lysate was assayed using the Bradford reagent, and 100 μg of protein sample were loaded on each lane of a 10% acrylamide SDS/PAGE gel. Nonspecific protein binding sites in the membranes were blocked with 5% milk in TBS supplemented with 0.05% Tween®-20, and incubated with an anti-cPLA2 mAb from Santa Cruz Biotechnology (sc-454). This was followed by incubation with horseradish peroxidase-conjugated goat anti-mouse IgG Ab. Detection was performed using the ECL system (Amersham Biosciences).

Assay for Phagocytosis and Flow Cytometry—Cells were incubated for different times with zymosan particles conjugated with Alexa Fluor® 488 at the concentration of 5 particles per cell at 37°C. Subsequently, cells were washed, treated with 100 units/ml lyseform in phosphate-buffered saline for 10 min at room temperature in order to dissolve extracellular zymosan, and then resuspended in 500 μl of phosphate-buffered saline supplemented with 1 mM EDTA for analysis by flow cytometry in a FACSscan® cytometer (Becton Dickinson, San Jose, CA). Parallel controls were performed at 4°C to block endothelial uptake of the particles. The surface display of CD11b was determined by indirect immunofluorescence with anti-human CD11b/Mac-1 IgG, mAb, followed by washing with PBS, and incubation with goat anti-mouse IgG-FITC conjugate (Sigma) 1:100 for 30 min at 4°C. Iso- matched irrelevant Ab was used as control.

RESULTS

Stimulation of Human Monocytes via Both FcγR and CR3 Induces [3H]AA Release—Previous studies in the human monocyte cell line THP-1 showed that stimulation of FcγR failed to release [3H]AA (19), thus suggesting that FcγRI and FcγRIIA, which are the types of FcγR receptors expressed in this cell line, do not trigger the cytosolic phospholipase A2 (cPLA2) route, i.e. the enzyme pathway involved in the selective release of AA from membrane phospholipids. To address whether this lack of response of the cPLA2 pathway was a general property of AA metabolism in THP-1 cells or whether this was restricted to some stimuli, THP-1 cells were activated with complement-coated zymosan particles, a stimulus resembling opsonized microbial agents, which induces AA release by acting through the β2 integrin Mac-1/CR3 (20). Under these conditions [3H]AA release was not observed (data not shown). Some experiments showed that THP-1 cells expressed CR3, as judged form the surface display of CD11b demonstrated by flow cytometry (Fig. 1A), and engulled complement-coated zymosan particles (Fig. 1, B and C). Taken together these findings suggest that THP-1 cells, which are a good model to study the responses involving transcriptional regulation and gene expression in response to FcγR cross-linking (21), may be activated by physiological stimuli without a concomitant release of AA, thus agreeing with the low amounts of eicosanoids yielded by these cells in the absence of phorbol ester- and dimethyl sulfoxide-induced differentiation (22). Since unlike monocytes, THP-1 cells do not show spontaneous adherence to plastic dishes, experiments were conducted in human monocytes to determine whether the absence of adherence-induced signals could account for the lack of FcγR-mediated AA release of THP-1 cells. As shown in Fig. 2, adherence of monocytes to plastic dishes is not a requirement for [3H]AA release, since non-adhered mononuclear cells released [3H]AA in response to both IC and opsonized zymosan. Because platelets may be present in mononuclear cell preparations and express FcγRIIA, some experiments were conducted on platelets to address their potential contribution to [3H]AA release. Addition of IC at the dose of 100 μg/ml for 90 min at 37°C to [3H]AA-labeled human platelets did not elicit [3H]AA release above that found in control cells (data not shown). Moreover, non-adhered mononuclear cells did not release [3H]AA in response to thrombin (Fig. 2), an agonist known to induce AA release in platelets, thus indicating that platelet contamination does not account for the release of [3H]AA elicited by IC on human mononuclear cells. To rule out the possible contribution to [3H]AA release of the lymphocytes present in the preparation of mononuclear cells, further experiments were carried out in adhered monocytes and monocyte-derived macrophages. As shown in Fig. 2, these cell populations released [3H]AA in response to both physiological and non-physiological agonists.
Since LPS is a potential contaminant of many biological reagents, the effect of concentrations of *Escherichia coli* LPS known to be active on the NF-κB route coupled to the TLR-4 system was addressed. LPS concentrations of 1 and 10 μg/ml did not elicit [3H]AA release on human monocytes, thus making it unlikely that contamination by LPS could account for the
effect of IC on [3H]AA release (Fig. 2). The temporal course of [3H]AA release elicited by IC was similar to that produced by opsonized zymosan particles (Fig. 3A). As regards the dose-dependence, maximal release was observed with 100 μg/ml IC, whereas higher concentrations induced the release of lower amounts of [3H]AA (Fig. 4A). In contrast, [3H]AA release induced by engagement of CR3 by opsonized zymosan particles plateaued at a concentration of 1 mg/ml and did not show significant changes at higher concentrations (Fig. 3B). To confirm that the enhancing effect of serum on the ability of zymosan particles to release [3H]AA was dependent on the activation of the complement system, some experiments were conducted using serum depleted of Ca²⁺ and Mg²⁺ by treatment with Chelex-100. As shown in Fig. 3B, this treatment did not modify the effect of zymosan particles, thus making it unlikely that serum components other than complement factors could account for the observed effect.

**Effect of the Covalent Linkage of C3bi to IgG on the Capacity of IC to Induce [3H]AA Release**—Since formation of IC in fluids containing complement is accompanied by the covalent linkage of C3bi onto the Ab and because C3bi coating of zymosan is known to increase AA release in leukocytes, some experiments were carried out with preformed IC treated with NHS to allow the formation of adducts between IgG γ-chain and C3b-α-chain, a phenomenon which has been related to the elimination of IC with a limited inflammatory response (4, 23). The formation of these complexes was verified by SDS-PAGE and confirmed by the sensitivity of the formation of these adducts to Mg²⁺ and Ca²⁺ chelation (Fig. 4C), and hydroxylamine treatment (Fig. 4D), as well as by immunoblotting with a polyclonal antihuman C3 Ab, which disclosed the presence of some protein bands (Fig. 4E), the Mᵣ of which was similar to that assigned to the Coomassie-stained protein bands.

Of note, the [3H]AA release induced by C3bi-IC was significantly lower than that induced by IC containing similar amounts of IgG (Fig. 4A), thus suggesting that the reaction of IC with C3bi gives rise to a IC lattice showing a distinct ability to interact with signaling receptors, most likely due to the ability of C3bi-IC to interact with CR3, in such way that it inhibits their ability to induce [3H]AA release through FcγR.

Treatment of C3bi-IC with anti-C3 IgG, but not with an irrelevant rabbit IgG, allowed the recovery of the ability to release [3H]AA (Fig. 4F), thus indicating that masking the C3bi moieties with IgG in the C3b-IC lattice makes these complexes similar to those formed in the absence of complement. Evidence of the involvement of the cPLA₂ route in the release of [3H]AA was obtained by addressing the appearance of the bandshift of the enzyme produced by phosphorylation on Ser-505 by MAP kinases. As shown in Fig. 4B, IC elicited a time-dependent phosphorylation of macrophage cPLA₂ which was slightly detectable 5 min after addition of the stimulus, but which did not reach its maximal extent until 30 min, thus agreeing with the protracted pattern of [3H]AA release observed.

Since these experiments suggested that the interaction of IgG with FcγR through the Fc portion is important for the induction of AA release, the effect of the removal of Fc portion on the ability of IC to release [3H]AA was addressed using OVA-coupled latex beads coated with anti-OVA F(ab)₂, taking advantage of the ability of F(ab)₂ to bind covalently to C3bi on the Ser-132 of the CH1 domain (24). Fig. 5A shows the characterization by SDS-PAGE of IgG-C3bi and F(ab)₂-C3bi ad-
ducts coupled to the latex-OVA beads. Fig. 5B shows the sensitivity of these adducts to hydroxylamine treatment as in the case of IgG-OVA IC. Moreover, immunoblotting with anti-C3 polyclonal Ab allows the detection of C3 associated to well characterized protein bands (Fig. 5C). Stimulation of monocytes with anti-OVA IgG coated latex-OVA beads produced a

FIG. 4. Release of [H]AA by monocytes stimulated with IC and C3bi-IC, phosphorylation of macrophage cPLA₂, characterization of C3bi-IC, and effect of C3bi masking on the ability of C3bi-IC to release [H]AA. A, adhered monocytes were incubated with different concentrations of IC and C3bi-IC for 90 min and the radioactivity released in the culture medium assayed. B, effect of IC on the phosphorylation of monocyte-derived macrophage cPLA₂ as judged from the shift of the protein band. C, IC were incubated with NHS in the presence or absence of Chelex-100, or in the presence of Mg²⁺ after removal of Chelex-100, and then washed prior to SDS-PAGE and staining with Coomassie Blue. D, effect of 1 M NH₂OH treatment on the ester bond between C3bi and IgG γ-chain. E, immunoblotting with a goat anti-human C3 Ab of IC treated with NHS. F, adhered monocytes were stimulated with 100 µg/ml of C3bi-IC, previously incubated with different concentrations of rabbit anti-human C3 IgG or with IgG purified from non-immune serum. The release of [H]AA into the medium was assayed 90 min after the addition of the stimuli. Data represent mean ± S.E. of four experiments with duplicate samples. G, SDS-PAGE analysis under reducing conditions of the fractions obtained by protein G Superose FPLC, which were used for the experiments represented in F. P-, phosphorylated; *, p < 0.05 as compared with the same amount of C3bi-IC. ***, p < 0.05 as compared with C3bi-IC treated with non-immune IgG.
significant [3H]AA release, whereas this was not observed when latex-OVA beads were coated with F(ab′)2 nor with latex-OVA beads coated with F(ab′)2 and coupled to C3bi, thereby indicating that Fc-FcγR interaction is essential for IC-induced [3H]AA release and that stimulation through C3bi does not elicit productive binding in this system (Fig. 5D).

Effect of Opsonic versus Non-opsonic Stimulation of Monocytes—β-Glucan shares with C3bi the ability to interact with CR3, although on different portions of the receptor molecule (20). Coating with C3bi significantly enhanced the ability of zymosan particles to release [3H]AA in human monocytes, as well as the phagocytic uptake of the particles by both THP-1 cells and monocyte-derived macrophages (Fig. 1, C and D), thus suggesting that contrary to IC, opsonization of zymosan particles may enhance their proinflammatory properties. Since cell receptors such as dectin-1 (25), a mannan-dependent receptor (26), and TLR-2 (27) have been related to the recognition of zymosan by leukocytes, the effect of several substances binding to those receptors was addressed. As shown in Fig. 6, mannan elicited significant [3H]AA release at concentrations above 5 mg/ml, this effect being additive with that produced by opsonized zymosan. In contrast, the soluble β-glucan laminarin,
which is a genuine ligand of dectin-1 (25), did not elicit [3H]AA release at the same range of concentrations. The effect of those substances on zymosan particle uptake was studied in mono-cyte-derived macrophages, in view of the robust particle uptake of these cells, as compared with monocytes. Mannan diminished the uptake of non-opsonized zymosan particles (mean fluorescence intensity 0.510 ± 0.03 AU, versus 1.01 ± 0.06 AU in control cells, p < 0.05, Fig. 7A, right upper panel), whereas laminarin and antibodies against CD11b, TLR-2, and TLR-4 lacked any significant effect as compared with an irrelevant rabbit Ab (mean fluorescence intensity 1.01 AU in control cells, versus 0.70–0.85 AU in the presence of the different additions), and C3bi-IC slightly enhanced zymosan uptake (1.19–1.44 AU, Fig. 7A, lower panels). When C3bi-coated zymosan particles were used, mannan even enhanced the fluorescence (Fig. 7B).

Treatment with anti-CD11b Ab and C3bi-IC produced a consistent inhibition of particle uptake (Fig. 7, B and C). In contrast, treatment with anti-TLR-2 Ab showed an inhibitory effect not significantly different from that produced by an irrelevant rabbit IgG Ab, anti-TLR-4 Ab, and non-opsonized IC (Fig. 7, B and C), thereby indicating that even though engulfment of zymosan particles may involve distinct types of receptors, the CR3 opsonic pathway seems the most relevant one in view of the enhanced uptake elicited by C3bi-coating, and the blockade elicited by anti-CD11b Ab and C3bi-IC.

**DISCUSSION**

The results herein presented show for the first time coupling of AA release to the FcyR signaling pathway in human mono-cytes. This agrees with similar properties assigned to the structurally related FcεRI expressed in mast cells, the cross-linking of which and the ensuing activation of the MAP kinase/cytosolic phospholipase A2 route lead to AA release (28). However, this finding is at variance with our previous studies using the THP-1 cell line (19), points to the existence of important differences in the behavior of peripheral blood mononuclear and monocytic cell lines, and suggests the existence of a variety of factors modulating the availability of AA and its likely influence by in vitro growing and cell division (29). Addressing the production of lipid mediators by Ab-driven mechanisms is a subject of pathophysiological relevance in view of the role played by these compounds in immune-mediated tissue injury, but the present findings may also be relevant to understand the process of maturation of monocyte-derived dendritic cells mediated by FcyR (30, 31), in view of the role played by cPLA2 in dendritic cell differentiation and function (32).

C3bi-coating of the IC allows interaction with CR3, a prototypic receptor of the innate immune response with binding capacity for a wide array of ligands, including Mycobacterium tuberculosis and Candida albicans (33). A consequence of this multiple binding capacity of CR3 is its ability to reach optimal conditions for triggering of phagocytosis and degranulation when C3bi and polysaccharides are recognized simultaneously (34, 35). On this basis, opsonized-zymosan particles and microbes may follow this paradigm of optimal productive binding, whereas other C3bi-containing particles such as C3bi-opsonized erythrocytes, the membranes of which do not bear CR3-binding polysaccharides, are bound by CR3 without stimulating phagocytosis and oxidative burst. Mapping of CR3 has shown that the interaction with C3bi and β-glucans occurs through two separate binding (35, 36). β-Glucan binding takes place on the lectin-like site on the C-terminal region of CD11b and C3bi binds to the I-domain. On this basis, it seems likely that the interaction of C3bi-IC with CR3 diverts the IC from productive interactions with FcyR in the absence of anchoring to the lectin-like site. This view is consistent with the experimental findings herein reported and carried out with C3bi-IC to expose C3bi and Fc concurrently, and zymosan particles to present β-glucan and C3bi in parallel. The complementary approach of removing Fc portions preserving C3bi with OVA-coated latex beads bound to F(ab')2, also agrees with this hypothesis.

In keeping with the different binding properties of both zymosan and opsonized zymosan to CR3, phagocytosis of both zymosan occurs through a biochemical mechanism involving pseudopodial extensions, Rac and Cdc42 activation, and the kinase activity of Hck. In contrast, phagocytosis of opsonized zymosan involves the Src protein tyrosine kinases for their adaptor function, but not for their kinase activity (37). These findings may explain the differential biological consequences that might follow CR3 engagement, such as delayed or enhanced apoptosis, activation or not of the NADPH oxidase, suppression of IL-12 and interferon-γ function (38), and activation or not of the AA cascade. It could be argued that the effect we attribute to complement coating of zymosan particles could be due to binding of other molecules rather than C3bi. In fact, the effect of NHS-treated zymosan on the activation of cPLA2 has been attributed to IgG-coating and related to the occupancy of FcRIIA and FcγRIII in human polymorphonuclear leukocytes (39). However, in that study only the phosphorylation of cPLA2 was addressed and there was no data regarding [3H]AA release under those conditions. This is a relevant issue, since phosphorylation of cPLA2 is a necessary, but not a sufficient condition for the activation of the AA cascade (19). Moreover, involvement of antibodies in CR3-mediated phagocytosis of some fungi has been attributed to the presence of IgA and IgM class antibodies in human sera (40). Since in the present study the effect of NHS on [3H]AA release by zymosan was only observed when the particles were treated under conditions that allow complement activation, i.e., in the presence of Mg2+, a role for Abs on the mediation of the effect of opsonized zymosan on [3H]AA release seems unlikely.

Recent findings regarding the receptors involved in zymosan binding have challenged the central role for CR3 in β-glucan binding, inasmuch as the C-type lectin dectin-1 has emerged as the main receptor involved in the recognition of zymosan and C. albicans (25). Dectin-1, in concert with TLR-2 and MyD88, conveys the cell response leading to NF-κB activation and cytokine production (41, 42) by a mechanism mimicking the complex process of ligand recognition in the innate immune response, whereas TLRs act as integrator of cell signaling rather than receptors (43). This mechanism seems most suitable to explain the responses to zymosan linked to the NF-κB route, which contrary to AA release, are observed in the absence of C3bi-coating (41, 42). A coherent explanation taking into account the aforementioned views and the strong dependence of

**FIG. 6. Effect of mannann and laminarin on the release of [3H]AA by monocytes.** Adhered monocytes were incubated for 90 min with the indicated additions in the presence or absence of 0.5 mg/ml of complement-coated zymosan particles. [3H]AA release was assayed at the end of this period. Data represent mean ± S.E. of four experiments in duplicate.
Fig. 7. Effect of different additions on zymosan uptake. A, effect of mannan and C3bi-IC on the uptake of zymosan particles conjugated with Alexa Fluor® 488 by monocyte-derived macrophages. Cells were incubated for 90 min at 37 °C in the presence of 5 particles of labeled zymosan. Histograms with thin tracing in the left upper panel represent cells treated at 4 °C to block the phagocytosis of the particles. Histograms with wide tracing in the right upper panel represent cells treated with a 50-fold excess of mannan. B, phagocytosis of C3bi-coated zymosan particles conjugated with Alexa Fluor® 488 by macrophages. In all cases, cells were incubated for 90 min at 37 °C in the presence of 5 particles of labeled zymosan and a 50-fold excess of mannan or laminarin as indicated. 10 µg/ml of the different Ab, IC or C3bi-IC. Thin tracing shows zymosan particle uptake in the absence of additions. Wide tracing represents zymosan uptake in the presence of the indicated additions. C, represents C3bi-coated zymosan particle uptake in the presence of the different additions expressed as mean fluorescence intensity in arbitrary units. Data represent mean ± S.E. of 4 independent experiments. *, p < 0.05; **, p < 0.01 as compared with the control.
zymosan binding on C3bi-coating, could be that C3bi-CR3 interaction are critical to promote contact between zymosan and the cell membrane, whereas AA release could be determined by other signaling molecules. In this connection, since mannan showed an additive effect on [3H]AA release with complement-coated zymosan, it seems likely that other cell receptor(s), for instance the mannose receptor, might also trigger the AA cascade, since mannosse may be present at a variable extent in zymosan particles (20). Since the high affinity ligand-binding state of CR3 triggered by “inside-out” integrin signaling can be achieved by activation of a variety of receptors and signaling pathways involving protein kinase C- and MAP kinase-dependent mechanisms, it seems likely that the wide array of responses elicited by zymosan particles, might emerge from the integration of signals from different receptors. In summary, the present data disclose a mechanism dependent on the complement system, which diverts IC formed during adaptive immune responses from triggering of the AA cascade to a CR3-dependent pathway with reduced AA release. On the contrary, binding of C3 by fungus-derived components under conditions mimicking an innate immune response enhances AA release.

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