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Serum Amyloid P Component and C-Reactive Protein Mediate Phagocytosis Through Murine FcγRs[1]

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The pentraxins, serum amyloid P component (SAP) and C-reactive protein (CRP) are acute-phase serum proteins in mice and humans, respectively. Although SAP binds to DNA and chromatin and affects clearance of these autoantigens, no specific receptor for SAP has been identified. CRP is an opsonin, and we have shown that it binds to FcγR. Mice deficient in FcγR were used to assess the role of these receptors in phagocytosis by pentraxins using zymosan as a ligand. Phagocytosis of zymosan by bone marrow macrophages (BMM) was enhanced by opsonization with SAP or CRP. BMM from mice deficient in all three FcγR or in γ-chain ingested unopsonized zymosan, but phagocytosis of SAP- or CRP-opsonized zymosan was not enhanced. SAP binding to BMM from γ-chain-deficient mice was also greatly reduced, indicating little or no binding of SAP to FcγRII. SAP and CRP opsonized zymosan for phagocytosis by BMM from mice deficient in FcγRII or FcγRIII. SAP, but not CRP, opsonized zymosan for uptake by neutrophils that express only low levels of FcγRI. Together these results indicate that FcγRI and FcγRIII are receptors for SAP in the mouse. Opsonization of zymosan by CRP is mediated through FcγRI. Pentraxins are major proteins of the innate immune system and arose earlier in evolution than Igs. The use of FcγR by the pentraxins links innate and adaptive immunity and may have important consequences for processing, presentation, and clearance of the self-Ags to which these proteins bind. ***The Journal of Immunology,*** **2001, 166: 1200–1205.**

Serum amyloid P component (SAP)[3] is a major acute-phase reactant in the mouse (1). Baseline levels of SAP in the blood vary markedly among different mouse strains. However, all mice examined to date appear to undergo a very marked up-regulation of SAP in response to inflammation (2). SAP is the precursor of tissue amyloid P component, a constituent of all types of amyloid. However, it is unlikely that this is the primary function of this protein, as it tends to stabilize the pathogenic deposition of amyloid proteins.

SAP and C-reactive protein (CRP) are members of the pentraxin family of proteins. These proteins are characterized by cyclic pentameric structure, sequence homology, and calcium-dependent interactions with ligands (3). Like other molecules of the innate immune system, SAP and CRP recognize ligands from necrotic and apoptotic cells as well as determinants on microorganisms. SAP binds to microbial polysaccharides and matrix components through carbohydrate determinants including heparin, 6-phosphorylated mannose, 3-sulfated saccharides, and the 4,6-cyclic pyruvate acetal of galactose (4, 5). Other physiological ligands of SAP include DNA, chromatin, and histones in cell nuclei as well as in solution (6). The interaction of SAP with nuclear Ags has been proposed to be a primary function of this molecule. This hypothesis is supported by findings in recently described SAP-deficient mice that spontaneously developed anti-nuclear Abs and glomerulonephritis (7). These studies, as well as earlier experiments in which SAP was injected or induced by an acute-phase stimulus (8), demonstrate a slower clearance of chromatin from the bloodstream in the presence of SAP. The reduced clearance rate of chromatin and nucleosomes in the presence of SAP was associated with increased uptake by the liver and decreased localization in the kidneys (8).

Despite these observations on the effects of SAP on chromatin clearance, there have been few studies of SAP binding to receptors on phagocytic cells and to our knowledge no demonstration that SAP can act as an opsonin. It has been reported that SAP binds to peritoneal macrophages from mice in a saturable manner (9). The binding was calcium dependent and inhibitable by mannose and mannose phosphates, leading the authors to conclude that the mannose 6-P receptor is the receptor for SAP. High- and low- affinity binding of SAP to human PMN has also been described (10). This binding was calcium dependent and could be inhibited by CRP and to a lesser extent by aggregated IgG.

We recently determined that CRP binds to both human and mouse receptors for IgG (FcγR) (11, 12). CRP has long been recognized as an opsonin, and like SAP it binds to both microbial determinants and components of damaged cells (13). Because of the similarities in structure and activities between SAP and CRP, we decided to determine whether SAP also binds to FcγR and to compare the receptors used by these two proteins for opsonization. We studied SAP and CRP opsonization of zymosan, because both SAP and CRP bind to zymosan and zymosan has been widely used in studies of phagocytosis.

Three classes of FcγR are present in mice (14). The high-affinity receptor for IgG, FcγRI, is expressed primarily on monocytes and macrophages in association with the γ-chain. Two low-affinity receptors for IgG have been described, FcγRII and FcγRIII. FcγRII is present on lymphocytes, macrophages, PMN, platelets, and mast

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[3] Abbreviations used in this paper: SAP, serum amyloid P component; BMM, bone marrow macrophages; CRP, C-reactive protein; GMFI, geometric mean fluorescence intensity; HSA, human serum albumin; PAB, PBS containing 0.1% BSA and 0.05% sodium azide; PEC, peritoneal exudate cells; PEGAM, PE-conjugated $F(ab')_2$ goat anti-mouse IgG; PI, phagocytic index; PMN, neutrophils; LCM, L cell conditioned medium.

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cells. Macrophages from mice expressing only FcγRII bind but do not ingest IgG-coated E (15). FcγRIII is also associated with the γ-chain and is expressed as a transmembrane protein on PMN, macrophages, NK cells, and mast cells. FcγRIII, like FcγRI, is capable of mediating phagocytosis. The relative contributions of the different FcγR classes to inflammation and phagocytosis have recently been elucidated by the development of mice genetically deficient in FcγR (15–17). These deficient mice were used in the current study to examine the role of FcγR in phagocytosis of zymosan opsonized by CRP or SAP.

The results demonstrate opsonization of zymosan by both SAP and CRP and that phagocytosis of SAP- and CRP-opsonized zymosan proceeds through phagocytic FcγR. In the case of CRP, phagocytosis proceeds through FcγRI only. However, phagocytosis of SAP-opsonized zymosan is mediated by either FcγRI or FcγRIII. These studies identify the phagocytic SAP receptors in the mouse and provide a possible mechanism for the SAP-mediated clearance of chromatin by Kupffer cells. They further point to distinct differences in specificity of CRP and SAP for FcγR. These results may help elucidate the common and disparate activities of CRP and SAP in the inflammatory response.

Materials and Methods

Reagents and Abs

FITC-zymosan was obtained from Molecular Probes (Eugene, OR). PE-conjugated $F(ab')_2$ goat anti-mouse IgG (PEGAM) and PE-conjugated $F(ab')_2$ goat anti-rat IgG were purchased from Caltag (Burlingame, CA). The hybridoma cell line producing rat mAb 2.4G2 was purchased from American Type Culture Collection (Manassas, VA), and the mAb was purified from culture supernatants by affinity chromatography on protein G-agarose. The mAb SAP-5, a murine IgG2a anti-human SAP, was purchased from Sigma (St. Louis, MO) and was used as ascites at 5 μg mAb/ml for immunofluorescence. Mouse IgG1 and IgG2a isotype control mAb were obtained from Sigma. Rat anti-mouse F4/80 (Serotec, Raleigh, NC) and Ly-6G (PharMingen, San Diego, CA) were used to identify macrophages and neutrophils (PMN) in peritoneal exudate cells (PEC) and bone marrow cell preparations. Mouse anti-sheep E Ab of the IgG2b isotype was obtained from Accurate Scientific (Westbury, NY). Sheep blood was obtained from Colorado Serum Company (Denver, CO). FMLP was obtained from Sigma.

Isolation of CRP and SAP

Human CRP was purified from pleural fluid by affinity chromatography and ion exchange chromatography as previously described (18). SAP was prepared as a side product of factor IX purification and generously provided to us by Dr. Walter Kisiel (Department of Pathology, University of New Mexico, Albuquerque, NM). Briefly, SAP was copurified with coagulation factor IX from Proplex by immunoaffinity chromatography as described (19). SAP was then separated from factor IX by Q Sepharose Fast Flow (Pharmacia, Piscataway, NJ) chromatography. Minor contaminants were removed from SAP by anion exchange chromatography on a Mono Q column using FPLC (Pharmacia, Piscataway, NJ) with a 0.15–0.5 M NaCl gradient in 20 mM Tris, pH 7.8 (20).

Mice

Male C57BL/6Ncr mice were purchased from the National Cancer Institute (Frederick, MD) and were used as controls for the γ-chain-deficient and FcγRIII-deficient mice. Male B6×129 F_2J mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and were used as controls for the FcγRII- and the γ-chain/FcγRII-deficient mice. Male γ-chain-deficient (15), FcγRII-deficient (17), and γ-chain/FcγRII-deficient mice were purchased from Taconic Farms (Westminster, NY). FcγRIII-deficient mice were purchased from The Jackson Laboratory. All mice were housed conventionally and were used between 3 and 5 mo of age. Paired cultures of bone marrow macrophages (BMM) from age-matched deficient and control mice were compared in each phagocytosis experiment. All animal studies were approved by the Veterans Affairs Animal Committee Review Board.

Cells

BMM were prepared as previously described (21). Briefly, mice were killed, femurs were isolated under sterile conditions, the ends of the femurs were excised and bone marrow was flushed from the femurs in HBSS with 0.2% human serum albumin (HSA). Cells were resuspended in 12–15 ml DMEM with 2% FBS and 2% L cell conditioned medium (LCM, a source of M-CSF) and allowed to adhere to tissue culture dishes for 2 h. Nonadherent cells were removed and cultured in tissue culture flasks in DMEM, 2% FBS, 15% LCM for 7–10 days. Nonadherent cells were removed after overnight culture, and cultures were fed after 4 days. BMM were removed from tissue culture flasks with 10 mM EDTA for use in binding or phagocytosis assays. BMM were resuspended in MEM, 2% FBS, 15% LCM with 1 mM $MnCl_2$ for phagocytosis assays or in PBS containing 0.1% BSA and 0.05% sodium azide (PAB) for staining.

Bone marrow PMN were purified from C57BL/6- and γ-chain-deficient mice using NIM-2 isolation medium (Cardinal Associates, Santa Fe, NM). Bone marrow leukocytes were resuspended in RPMI 1640 with 10 mM HEPES, 5% FCS, and 1 mM $MnCl_2$ at 4×10^6 PMN/ml. The percentage of PMN was determined by cresyl violet staining and was 49% for wild-type and 48% for deficient mice.

Elicited PEC were obtained by peritoneal lavage of mice injected with 1 ml of 3% thioglycollate medium (Difco, Detroit, MI) 5 days prior to harvest. Cells were collected in 10 ml of HBSS without calcium and magnesium. PEC prepared in this way were >50% macrophages determined by staining with DiffQuik (Dade International, Miami, FL).

Opsonization

Sheep E were opsonized with mouse IgG2b or IgG2a monoclonal anti-sheep E as previously described (22), washed, and resuspended at 5×10^8/ml in dextrose-gelatin Veronal-buffered saline. FITC-zymosan was incubated for 30 min at 37°C with 100 μg/ml SAP or CRP in HBSS containing $CaCl_2$ and $MgCl_2$, washed, and resuspended at 4×10^8/ml in the same buffer.

Phagocytosis assays

To measure phagocytosis 4×10^5 BMM or PMN in 100 μl culture medium were centrifuged briefly (1 min at 200 × *g*) with opsonized FITC-zymosan (2×10^6/tube for BMM and 4×10^6/tube for PMN) and incubated for 30 min at 37°C. The fluorescence of the noningested yeast particles was quenched by the addition of trypan blue to a final concentration of 0.02%. Phagocytosis was scored visually on a Zeiss Axiovert fluorescent microscope. The results are expressed as the phagocytic index (PI) (number of ingested yeast cells or E per 100 BMM or PMN). For E-IgG2b or E-IgG2a, 7.5×10^6/tube were added, and noningested E were lysed prior to analyzing phagocytosis by phase microscopy.

The relative PI was calculated by dividing the PI of opsonized zymosan by the PI of unopsonized zymosan. Mean values of different mouse strains were compared by *t* tests.

SAP and CRP binding assays

BMM were released from culture flasks with EDTA, washed, and incubated with the indicated concentrations of SAP in PAB for 1 h on ice. Cells were washed twice with PAB and incubated with mAb SAP-5 or rat anti-mouse F4/80. Following a 30-min incubation on ice, cells were washed twice and incubated for 30 min with PE-conjugated secondary Abs. Cells were washed twice, resuspended in PAB, and analyzed on a Becton Dickinson FACScalibur flow cytometer (Mountain View, CA). For inhibition experiments, heat-aggregated human IgG (63°C for 45 min) was added to cells during the incubation with SAP. Forward and side scatter characteristics of BMM were determined by F4/80 binding and used to collect cells for fluorescent analysis.

Zymosan was washed with HBSS (containing calcium and magnesium), counted, and distributed into tubes. Zymosan (10^6 particles/tube) was incubated with the indicated concentrations of CRP or SAP for 30 min at 37°C. Zymosan was washed and binding determined by incubation with 5 μg/ml mAb (2C10 for CRP and SAP-5 for SAP) for 30 min on ice. After washing, zymosan was incubated with PEGAM and analyzed on a Becton Dickinson FACScalibur flow cytometer.

Fluorescence was analyzed using CellQuest software (Becton Dickinson). Fluorescence data were collected on a log scale, and data are reported as the difference in geometric mean fluorescent intensity (GMFI) in the presence of CRP or SAP compared with mAb and PEGAM alone. Binding curves were generated by nonlinear regression analysis using GraphPad Prism software (GraphPad Software, San Diego, CA).

Results

SAP and CRP binding to zymosan

As SAP closely resembles CRP in both structural and binding characteristics and because we have recently demonstrated that

CRP binds to FcγR, we decided to test whether SAP was capable of enhancing the phagocytosis of zymosan. Although previous studies have demonstrated the ability of both CRP and SAP to bind to zymosan, the optimal concentrations for binding of the two pentraxins were unknown. Therefore, preliminary studies were performed to examine the binding of SAP and CRP to zymosan (Fig. 1). SAP and CRP bound to zymosan with similar avidity. The total amount of pentraxin bound at saturation was comparable. The normal concentration of SAP in human serum is ~50 μg/ml (23), and CRP concentrations range from <5 μg/ml at baseline to >200 μg/ml during an acute-phase response (24). For subsequent experiments, FITC-zymosan was opsonized with 100 μg/ml SAP or CRP.

Enhanced phagocytosis of SAP-opsonized zymosan requires FcγR

Pretreatment of FITC-zymosan with different concentrations of SAP enhanced its phagocytosis by BMM in a dose-dependent manner (data not shown). Maximal enhancement of phagocytosis was seen at ~50 μg/ml SAP, consistent with the binding data. To determine whether FcγR were involved in the phagocytosis of particles opsonized by SAP, BMM from various strains of FcγR-deficient mice were tested. BMM from mice deficient in all three FcγR were cultured in parallel with BMM from control mice. FITC-zymosan was opsonized with 100 μg/ml of SAP or buffer, and phagocytosis was measured (Fig. 2). Uptake of zymosan by BMM from wild-type mice was enhanced following opsonization with SAP. BMM from FcγR-deficient mice ingested unopsonized zymosan as efficiently as wild-type BMM, but opsonization with SAP did not enhance ingestion. Significant phagocytosis of unopsonized zymosan by BMM occurred due to the uptake of zymosan through other receptors. However, SAP enhanced the level of phagocytosis of zymosan by BMM from wild-type but not FcγR-deficient mice in three experiments.

To determine which FcγR were most important for the uptake of opsonized zymosan, three additional strains of mice were tested. First, phagocytosis of zymosan was examined in γ-chain-deficient mice. These mice do not express FcγRI or FcγRIII, both of which require the γ-chain for expression. Macrophages from γ-chain-deficient mice express only FcγRII and do not phagocytose E-IgG (15). As expected, these mice also showed markedly decreased phagocytosis of SAP-opsonized zymosan (Fig. 3). The ability of CRP to opsonize FITC-zymosan was examined in the same assay. Consistent with our previous identification of FcγRI and FcγRII as CRP receptors in the mouse (12), CRP opsonized zymosan for phagocytosis by wild-type but not γ-chain-deficient BMM.

The phagocytosis of SAP- and CRP-opsonized FITC-zymosan was also examined using BMM from FcγRII-deficient mice. Enhanced phagocytosis of FITC-zymosan mediated by either SAP or CRP was unchanged compared with wild-type controls (Fig. 4).

A statistical comparison of the ability of SAP to opsonize zymosan for uptake by BMM from normal and FcγR-deficient mice is shown in Table I.

SAP binding to mouse macrophages requires γ-chain expression

SAP binding to wild-type and γ-chain-deficient BMM was determined by flow cytometry using mAb SAP-5 and PEGAM (Fig. 5). The apparent K_D of the interaction of SAP with wild-type BMM determined by nonlinear regression analysis of the binding curve was $\sim 7.5 \times 10^{-7}$ M. BMM from γ-chain-deficient mice bound much less SAP than did wild-type BMM. SAP binding to F4/80-positive PEC from γ-chain-deficient mice was also greatly reduced compared with the same PEC from wild-type mice (data not shown). The binding of 50 μg/ml SAP to PEC was inhibited

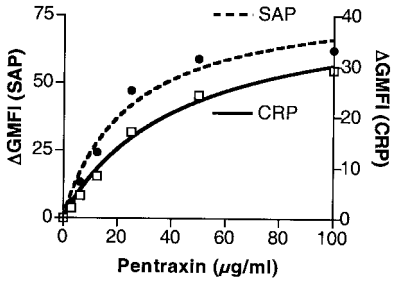

FIGURE 1. SAP and CRP binding to zymosan. Zymosan was incubated with the indicated concentrations of SAP or CRP for 30 min at 37°C, washed, and binding was detected using anti-SAP mAb SAP-5 or anti-CRP mAb 2C10 and PEGAM. The GMFI was determined by flow cytometry. Results from a representative experiment are shown.

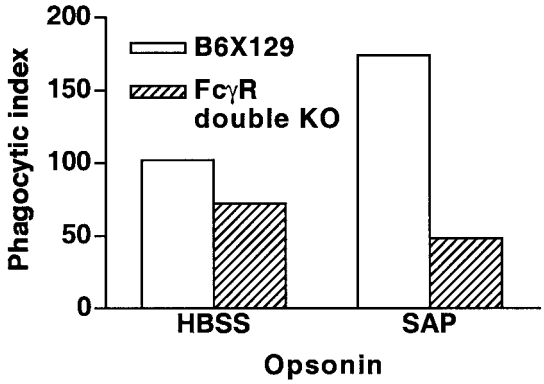

FIGURE 2. SAP does not opsonize zymosan for phagocytosis by BMM from FcγR-deficient mice. FITC-zymosan was incubated with 100 μg/ml SAP or HBSS for 30 min at 37°C and then washed once with HBSS. Opsonized zymosan was then incubated with BMM at a ratio of 4:1. BMM and FITC-zymosan were centrifuged to enhance contact and incubated for 30 min at 37°C. Trypan blue was added to quench the fluorescence of uningested FITC-zymosan. Phagocytosis was scored by fluorescent microscopy. Results are expressed as the number of FITC-zymosan per 100 BMM (PI). Results shown are representative of four separate experiments done with paired wild-type and deficient BMM.

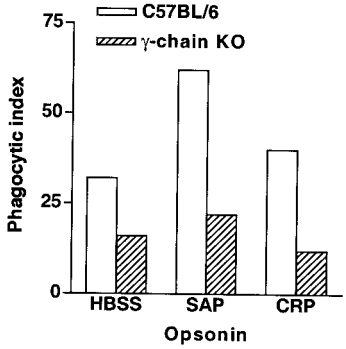

FIGURE 3. SAP and CRP do not opsonize zymosan for phagocytosis by BMM from γ-chain-deficient mice. FITC-zymosan was incubated with 100 μg/ml SAP or CRP or with HBSS for 30 min at 37°C and then washed once with HBSS. Opsonized zymosan was then incubated with BMM at a ratio of 4:1. BMM and FITC-zymosan were centrifuged to enhance contact and incubated for 30 min at 37°C. Trypan blue was added to quench the fluorescence of uningested FITC-zymosan. Phagocytosis was scored by fluorescent microscopy. Results are expressed as the number of FITC-zymosan per 100 BMM (PI). A representative experiment (of three) is shown.

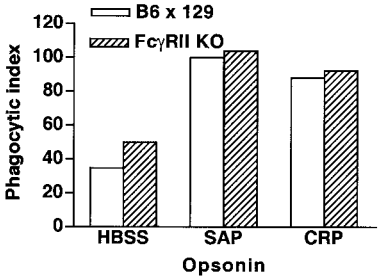

FIGURE 4. FcγRII-deficient mice show no decrease in the level of phagocytosis of FITC-zymosan opsonized by SAP or CRP. FITC-zymosan was incubated with 100 μg/ml SAP or CRP or with HBSS for 30 min at 37°C and then washed once with HBSS. Opsonized zymosan was then incubated with BMM at a ratio of 4:1. BMM and FITC-zymosan were centrifuged to enhance contact and incubated for 30 min at 37°C. Trypan blue was added to quench the fluorescence of uningested FITC-zymosan. Phagocytosis was scored by fluorescent microscopy. Results are expressed as the number of FITC-zymosan per 100 BMM (PI). A representative experiment (of two) is shown.

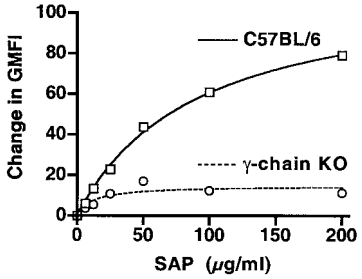

FIGURE 5. BMM from γ-chain-deficient mice show decreased SAP binding. BMM from C57BL/6 mice or γ-chain-deficient mice were incubated with SAP in PAB for 60 min on ice. SAP binding was detected using mAb SAP-5 and PEGAM. The increase in GMFI relative to cells incubated with mAb and PEGAM is shown.

>95% in the presence of 100 μg/ml aggregated IgG, further supporting a requirement for FcγR in SAP binding. These results differ from previous binding studies using CRP in which significant binding to peritoneal macrophages from γ-chain-deficient mice was observed indicating binding to FcγRII (12). Thus, SAP shows dose-dependent and saturable binding to mouse macrophages with a moderate affinity. This interaction requires one or both receptors associated with the γ-chain. Because SAP is multivalent and its binding to microbial cells occurs through highly repetitive determinants on the surface, the physiological interaction between SAP and cellular receptors is likely to involve multiple receptors resulting in increased avidity of binding.

Enhanced phagocytosis of SAP- and CRP-opsonized zymosan can be mediated by FcγRI

Both FcγRI and FcγRIII are capable of mediating phagocytosis in vivo, and there is evidence that FcγRIII may be more efficient than FcγRI (25). To examine the role of FcγRI, the ability of BMM derived from FcγRIII-deficient mice to phagocytose zymosan was examined. As shown in Fig. 6 and Table I, BMM from these mice showed little or no decrease in their ability to phagocytose zymosan opsonized by SAP or CRP. These results suggest that the phagocytosis of zymosan via SAP and CRP can be mediated by FcγRI. Whether FcγRIII also contributes to the uptake of zymosan opsonized with SAP or CRP cannot be determined from this experiment. Soluble IgG1, IgG2a, and the rat mAb 2.4G2 to mouse FcγRII/III all failed to inhibit SAP-mediated phagocytosis by wild-type BMM (data not shown).

The ability of FcγRI to mediate phagocytosis of CRP- or SAP-opsonized zymosan was also examined by selective digestion of FcγRI from BMM with trypsin. Trypsin has been shown to selectively remove FcγRI from mouse macrophages (26). BMM were treated with 20 μg/ml trypsin for 30 min at 37°C, and phagocytosis of zymosan, opsonized zymosan and E-IgG2a was tested (26). The phagocytosis of E-IgG2a was decreased by 70%, indicating a loss of FcγRI, whereas ingestion of unopsonized zymosan was unchanged, indicating that general phagocytic responses were not affected. With this loss of FcγRI, the phagocytosis of CRP-zymosan was decreased by 55% and the phagocytosis of SAP-zymosan was decreased by 24%. Thus, CRP-mediated opsonization of zymosan appeared to be dependent on the presence of FcγRI, whereas SAP-mediated opsonization was less affected. As FcγRII is not a phagocytic receptor on mouse BMM, it appears that phagocytosis of SAP-opsonized zymosan may proceed through either FcγRI or FcγRIII.

Enhanced phagocytosis of SAP- but not CRP-opsonized zymosan can be mediated by FcγRIII

As FcγRI α-chain-deficient mice have only recently been produced and were not available for these studies, we tested the phagocytosis of zymosan by PMN, which express low levels of

Table I. *Comparison of phagocytosis of SAP-opsonized zymosan between mouse strains*

Mouse Strain	Relative PI[a] (Mean)	SEM	*n*	*p*[b]
C57BL/6	1.97	0.23	5	–
γ-chain knockout	1.20	0.14	3	0.0055
FcγRIII knockout	1.68	0.11	3	0.31
B6 × 129	1.71	0.09	4	–
Double knockout	0.62	0.14	4	<0.0001
FcγRII knockout	1.81	0.26	2	0.59

[a] The relative PI (PI SAP-zymosan/PI unopsonized zymosan) for SAP-opsonized zymosan compared to unopsonized zymosan. PI for unopsonized zymosan were not significantly different for γ-chain knockout or double knockout mice compared to control mice.

[b] Values of *p* were determined by *t* tests between the deficient strain and the appropriate control strain.

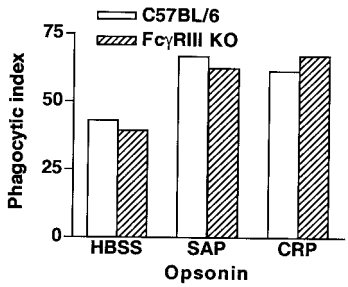

FIGURE 6. FcγRIII-deficient mice show no decrease in the level of phagocytosis of FITC-zymosan opsonized by SAP or CRP. FITC-zymosan was incubated with 100 μg/ml SAP or CRP or with HBSS for 30 min at 37°C and then washed once with HBSS. Opsonized zymosan was then incubated with BMM at a ratio of 4:1. BMM and FITC-zymosan were centrifuged to enhance contact and incubated for 30 min at 37°C. Trypan blue was added to quench the fluorescence of uningested FITC-zymosan. Phagocytosis was scored by fluorescent microscopy. Results are expressed as the number of FITC-zymosan per 100 BMM (PI). A representative experiment (of three) is shown.

FcγRI, to further examine whether SAP- or CRP-opsonized zymosan could be taken up through FcγRIII. Bone marrow PMN bound very low levels of monomeric IgG2a and did not ingest E-IgG2a, indicating a lack of functional FcγRI on these cells (data not shown). PMN were isolated from bone marrow and incubated with SAP- or CRP-opsonized zymosan or E-IgG2b as a positive control for FcγRIII-mediated phagocytosis. The ability of SAP and CRP to opsonize zymosan for phagocytosis by PMN from wild-type and γ-chain-deficient mice was tested. Phagocytosis was also examined in the presence of FMLP, which enhances phagocytosis through FcγR (22). As shown in Fig. 7, ingestion of E-IgG2b and SAP-zymosan by PMN was greater for wild-type compared with γ-chain-deficient mice, indicating that phagocytosis was mediated by FcγRIII. In contrast to the results with BMM (Fig. 3), CRP-zymosan was poorly ingested by PMN, and there was no difference between PMN from control and γ-chain-deficient mice. This finding is consistent with the inability of CRP to bind to mouse FcγRIII and indicates that there is insufficient FcγRI present to mediate phagocytosis. Similar results were seen in the presence of FMLP, with higher levels of phagocytosis for E-IgG2b and SAP-zymosan, but no increase in the uptake of CRP-zymosan or unopsonized zymosan. Thus these results indicate that SAP can opsonize zymosan for phagocytosis through either FcγRIII (Fig. 7) or FcγRI (Fig. 6).

Discussion

The major findings reported here are first that SAP is an efficient opsonin for zymosan and second that SAP-mediated phagocytosis is dependent on the presence of receptors for IgG on mouse phagocytic cells. Another significant finding reported for the first time here is that opsonization for phagocytosis by CRP occurs through FcγR. Although CRP-enhanced phagocytosis was previously demonstrated in murine (27) and human phagocytes (28) a unique receptor was felt to be responsible (27). These studies suggest that both CRP and SAP are opsonins that act through specific binding through FcγR on phagocytic cells.

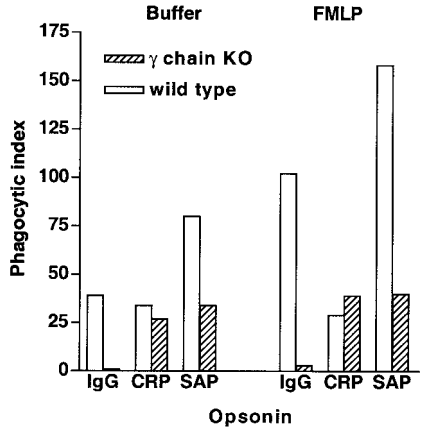

FIGURE 7. SAP enhances phagocytosis of FITC-zymosan by PMN from C57BL/6 but not γ-chain-deficient mice. Bone marrow PMN were isolated on NIM-2 medium and resuspended in medium. In the FMLP-stimulated group, FMLP was added at 100 μM. FITC-zymosan was incubated with 100 μg/ml SAP or CRP for 30 min at 37°C and then washed once with HBSS. Opsonized FITC-zymosan or E-IgG2b was then added to PMN at a ratio of 10:1. The tubes were centrifuged to enhance contact and incubated for 30 min at 37°C. Uningested E-IGg2b were lysed. Trypan blue was added to quench the fluorescence of uningested FITC-zymosan. Phagocytosis was scored by microscopy. Results are expressed as the number of FITC-zymosan or E-IgG2b per 100 PMN (PI). A representative experiment is shown.

SAP has been demonstrated to bind to a wide variety of ligands in vitro including heparin, laminin (29), heparan sulfate (30), fibronectin, and C4b binding protein (31) as well as a number of microbial polysaccharides (4, 5). The binding site is thought to be the calcium-dependent ligand binding site that specifically recognizes phosphoethanolamine. The relative physiological significance of these interactions is not known. SAP also binds to chromatin and DNA in the circulation (6). Recently, SAP has been shown to be an important mediator of the clearance and processing of chromatin (8). The development of autoantibodies to chromatin in SAP-deficient mice suggests an important role for this protein in the prevention of autoimmunity (7).

Saturable binding of SAP to phagocytic cells was previously demonstrated but the nature of the receptor was not identified (9, 10). Contrary to CRP, for which evidence of opsonic activity was presented early on, studies of the ability of SAP to enhance phagocytosis of bacterial or fungal pathogens are limited.

We have previously shown that CRP interacts with FcγRI and FcγRII in the mouse (12). Therefore, we wanted to determine whether the receptor for SAP was also an FcγR. The present results confirm that both SAP and CRP opsonize zymosan for phagocytosis by mouse BMM and that FcγR are required. The interaction of SAP with mouse FcγR differs from that of CRP. Both CRP and SAP are capable of binding FcγRI and FcγRI may be important for phagocytosis through both pentraxins. However, SAP was capable of enhancing phagocytosis in PMN, which express only low levels of FcγRI and in BMM after selective removal of FcγRI by trypsin, suggesting that FcγRIII is sufficient for SAP-mediated opsonization. Furthermore, SAP-mediated phagocytosis was enhanced by FMLP treatment of PMN. FMLP preferentially enhances phagocytosis through FcγR as opposed to complement receptors (22). This finding supports the role of FcγR in opsonization by SAP. Ongoing studies to be presented separately suggest that similar differences in FcγR binding specificity occur in the human as well. It is likely that these differences in receptor specificity will be reflected in different activities of SAP and CRP in vivo.

SAP binding was γ-chain dependent and inhibited by aggregated IgG, consistent with binding to FcγRI and FcγRIII. Both of these receptors can invoke a stimulatory response by the cell and are therefore likely to enhance the inflammatory response in the mouse. CRP, but not SAP, binds to FcγRII as well (12). FcγRII in mice is known to provide an anti-inflammatory signal through the recruitment of the inositol phosphatase Src homology 2 domain-containing inositol phosphatase (SHIP) (32). Although CRP binds to FcγRII, no increase in uptake of CRP-opsonized zymosan by BMM was observed in FcγRII-deficient mice. This is in contrast to the enhanced phagocytosis of E-IgG2b by PEC from FcγRII-deficient mice (33). These results may be explained by the interaction of CRP with FcγRI, whereas phagocytosis of E-IgG2b is predominately mediated by FcγRIII.

The interaction of SAP with phagocytic receptors, its ability to activate complement, and its abundance in the serum suggest that SAP may play a role in host defense as well. SAP has been shown to bind a number of human pathogens, especially fungal organisms. Singh et al. (34) reported that SAP enhanced the ability of mouse macrophages to kill *Listeria monocytogenes*. This listericidal activity was not associated with enhanced ingestion of the bacteria by SAP or with SAP binding to *Listeria*. Thus the nature of this effect was not determined. CRP was previously shown to provide protection from infection with *Streptococcus pneumoniae* (35).

It is now known that both CRP and SAP bind to FcγR in the human and the mouse. The evolutionary significance of these findings is of considerable interest as the pentraxins appeared earlier in evolution than the Igs. The pentraxins are present in the horseshoe crab, whereas the Igs do not appear until the jawed fishes (36). Therefore, it is possible that Fc receptors preceded the appearance of Ig and served as receptors for pentraxins.

The interaction of CRP and SAP, which are both capable of interacting with altered self and with microorganisms, identify the pentraxins as innate immune recognition molecules. However, the finding that they interact with FcγR bridges the gap between innate and acquired immunity. This connection may have served to facilitate the development of the adaptive immune system. It remains to be determined whether SAP and CRP are most important for the recognition of foreign Ags as exemplified by the protection afforded mice by CRP (35) or whether their role in regulating autoimmunity as suggested by studies of CRP and SAP in down-regulating the autoimmune response in systemic lupus erythematosus is more important (7, 37).

The function of SAP has long remained unknown despite extensive studies of its binding activities, its interaction with the complement system, and its role in the pathogenesis of amyloidosis. The finding that SAP can specifically interact with FcγR should help to delineate the in vivo functions of this ancient member of the innate immune system.

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