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Alessia Cotena, Virginia Maina, Marina Sironi, Barbara Bottazzi, Pascale Jeannin, Annunciata Vecchi, Nathalie Corvaia, Mohamed R. Daha, Alberto Mantovani and Cecilia Garlanda

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Complement Dependent Amplification of the Innate Response to a Cognate Microbial Ligand by the Long Pentraxin PTX3¹

Alessia Cotena,^{2*†} Virginia Maina,^{2*†} Marina Sironi,^{*} Barbara Bottazzi,^{*} Pascale Jeannin,[‡] Annunciata Vecchi,^{*} Nathalie Corvaia,[§] Mohamed R. Daha,^{||} Alberto Mantovani,^{3*†} and Cecilia Garlanda^{*}

The long pentraxin PTX3 is a fluid-phase pattern recognition receptor, which plays a nonredundant role in resistance against selected pathogens. PTX3 has properties similar to Abs; its production is induced by pathogen recognition, it recognizes microbial moieties, activates complement, and facilitates cellular recognition by phagocytes. The mechanisms responsible for the effector function of PTX3 *in vivo* have not been elucidated. OmpA, a major outer membrane protein of Gram-negative *Enterobacteriaceae*, is a microbial moiety recognized by PTX3. In the air pouch model, KpOmpA induces an inflammatory response, which is amplified by coadministration of PTX3 in terms of leukocyte recruitment and proinflammatory cytokine production. PTX3 did not affect the inflammatory response to LPS, a microbial moiety not recognized by PTX3. As PTX3 binds to C1q and modulates the activation of the complement cascade, we assessed the involvement of complement in the amplification of the response elicited by KpOmpA and PTX3. Experiments performed using cobra venom factor, C1-esterase inhibitor, and soluble complement receptor 1 indicate that PTX3 amplifies the inflammatory response to KpOmpA through complement activation. The results reported here demonstrate that PTX3 activates a complement-dependent humoral amplification loop of the innate response to a microbial ligand. *The Journal of Immunology*, 2007, 179: 6311–6317.

Innate immunity provides the first line of defense against invading pathogens and plays a key role in the activation and orientation of adaptive immune responses through the activity of cellular and soluble pattern recognition receptors (PRRs)⁴. Fluid phase PRRs include collectins, ficolins, and pentraxins (1, 2). The main and best-known functional roles of soluble PRRs are associated to their opsonic activity, to their involvement in complement activation and regulation of cell function (3).

Pentaxins, a superfamily of evolutionarily conserved soluble PRRs characterized by cyclic multimeric structures are divided in two subfamilies, the short and long pentraxins (2). The classical short pentraxins C-reactive protein and serum amyloid P component are acute phase proteins in human and mouse, respectively, produced in the liver in response to proinflammatory mediators,

most prominently IL-6 (4). Long pentraxins share similarities with the classical short pentraxins but differ for the presence of an unrelated long N-terminal domain, as well as for gene organization, chromosomal localization, cellular source, inducing stimuli and recognized ligands (5).

PTX3, the first long pentraxin identified (6), is rapidly produced by different cell types, which include myeloid dendritic cells, that are major producers of PTX3, mononuclear phagocytes, endothelial cells, smooth muscle cells, fibroblasts, and synovial cells upon stimulation with proinflammatory mediators like IL-1, TNF- α , microbial moieties, and agonists for different members of the TLR family (7, 8). Similarly to ligand-complexed C-reactive protein and serum amyloid P component (9), immobilized PTX3 was shown to bind with high affinity to C1q and activate the classical complement pathway, as assessed by C4 and C3 deposition (10, 11). Furthermore, PTX3 binds selected microorganisms, microbial moieties, the extracellular matrix component TNF α induced protein 6 (TNFAIP6 or TSG-6), and the angiogenic factor FGF2 (12–16). Recent studies (12, 13, 15, 17) with *ptx3*-deficient mice have shown that through these interactions, PTX3 plays complex non-redundant functions *in vivo*, ranging from innate immunity against specific microorganisms to the assembly of a hyaluronic acid-rich extracellular matrix and female fertility.

Outer membrane protein A (OmpA) belongs to a class of cell wall proteins, highly conserved among the *Enterobacteriaceae* and essential for bacterial integrity and virulence, which binds to and activates APCs (18). Recombinant OmpA from *Klebsiella pneumoniae* (KpOmpA) is recognized by the scavenger receptors LOX-1 and SREC-I, which cooperate with TLR2 in triggering cellular responses (14). The functional program activated by KpOmpA through TLR2 includes the production of PTX3, which, in turn, binds with high affinity to KpOmpA. KpOmpA-elicited *in vivo* inflammation is dependent on TLR2 activity and also is amplified by PTX3. These results indicated the relevance of the collaboration between the humoral (PTX3) and cellular (TLR2,

*Istituto Clinico Humanitas, Rozzano (Milan), Italy; [†]Institute of General Pathology, University of Milan, Italy; [‡]Institut National de la Santé et de la Recherche Médicale U564 and Laboratoire de Immunologie et Allergologie, University Hospital of Angers, Angers, France; [§]Centre de Immunologie Pierre Fabre, Saint Julien Genevois, France; and ^{||}Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands

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² A.C., and V.M., equally contributed to this paper.

³ Address correspondence and reprint requests to Dr. Alberto Mantovani, Istituto Clinico Humanitas, Via Manzoni 56, Rozzano, Italy. E-mail address: alberto.mantovani@humanitas.it

⁴ Abbreviations used in this paper: PRR, pattern recognition receptor; OmpA, outer membrane protein A; KpOmpA, *Klebsiella pneumoniae* OmpA; C1-INH, C1 esterase inhibitor; sC1r, soluble complement receptor 1; CVF, cobra venom factor.

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SREC, and LOX1) pattern recognition receptors in the innate immune response to the microbial moiety KpOmpA (14). Accordingly, PTX3 deficient mice show altered susceptibility to *Klebsiella pneumoniae* (19).

The mechanisms involved in the effector function of PTX3 *in vivo* have not been elucidated. *In vitro*, PTX3 has opsonic activity, facilitating recognition and ingestion of microbes by phagocytes (12, 13, 15, 17). Moreover, PTX3 activates the classic complement pathway (10, 11). It was, therefore, important to explore the mechanism(s) involved in the amplification of the innate response to microbial moieties by PTX3. In this study, we report that PTX3 amplifies the inflammatory response elicited by a cognate microbial ligand (OmpA) but not by LPS. The effect of PTX3 is associated with increased production of inflammatory mediators. Inactivation of complement with cobra venom factor (CVF), or inhibition by C1 esterase inhibitor (C1-INH) and soluble complement receptor 1 (sCR1) drastically reduced the effect of PTX3. Thus, PTX3 activates a complement-dependent pathway of amplification of the innate immune response to cognate microbial ligands.

Materials and Methods

Animals

129/Sv mice were obtained from Charles River Laboratories. *Ptx3*-deficient mice on 129/Sv background were generated by homologous recombination as described (12).

Procedures involving animals and their care were conformed with institutional guidelines in compliance with national (4D.L. N.116, G.U., suppl. 40, 18-2-1992) and international law and policies (EEC Council Directive 86/609, OJ L 358,1,12-121987; National Institutes of Health Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996). All efforts were made to minimize the number of animals used and their suffering.

Reagents

Recombinant human PTX3 was purified from Chinese hamster ovary cells (CHO) constitutively expressing the protein as described previously (10). Recombinant KpOmpA was expressed and purified by Pierre Fabre as described (20). Cobra venom factor (CVF) was obtained from Quidel. C1-INH (1U corresponded to the activity of 1 ml of normal plasma) was from Baxter-Immuno. sCR1 was from AVANT Immunotherapeutics. LPS from *E. coli* strain 055:B5 was obtained from Sigma-Aldrich. IL-1 β was obtained from Dompé and carboxymethylcellulose was obtained from Sigma-Aldrich.

Recombinant PTX3 and KpOmpA contained <0.125 endotoxin U/ml as checked by the *Limulus* amoebocyte lysate assay (BioWhittaker).

The 5-lipoxygenase inhibitor MK 886 was obtained from Cayman Chemicals and the cyclooxygenase inhibitor indomethacine was from Sigma-Aldrich.

Air pouch model

s.c. dorsal pouches were created by injection of 5 ml of sterile air followed, 3 days later, by a second injection of 3 ml of air. On day 6, 5–7 mice per experimental group received in the pouch in 1 ml of Dulbecco's (Ca²⁺/Mg²⁺) PBS, either KpOmpA (0.2–25 μ g/mouse), PTX3 (1–25 μ g/mouse), LPS (200 ng/mouse), IL-1 (20 ng/mouse), or the combination of PTX3 and the inflammatory stimuli preincubated in Ca²⁺/Mg²⁺PBS for 30 min at room temperature before injection. For the experiment with IL-1, the vehicle was carboxymethylcellulose 0.5% in Ca²⁺/Mg²⁺ PBS. At the indicated time points, mice were sacrificed and air pouches washed with 2 ml of ice cold saline. The lavage fluids were cooled on ice, the cells recovered and counted. Supernatants were harvested and stored at –80°C for further cytokines quantification. Cellular morphology was evaluated on cytopspins followed by Diff-Quick staining (Dade). When indicated, CVF (5 U/mouse), or MK 886 (1 mg/kg) and indomethacine (5 mg/kg) were administered i.p. 24 h or 1h, respectively, prior dorsal s.c. injection of the stimulus. C1-INH (15U/mouse) and sCR1 (10 mg/kg) were administered iv 1h before or simultaneously to OmpA injection, respectively.

Measurement of cytokines, chemokines, PTX3, and C3

Levels of murine IL-6 and JE/CCL2 in air pouch lavage exudates were determined using specific ELISA (R&D Systems) according to the manufacturer's protocols. Levels of PTX3 were measured by a sandwich ELISA as described (21, 22), using a mAb anti-mouse PTX3 (2C3), followed by a biotin conjugated mAb anti-mouse PTX3 (6B11).

Classical pathway activity up to C3 was assessed in a C3 deposition assay (23) using Maxisorb plates (Nunc) coated with human IgM (3 μ g/ml). Serum samples were diluted in BVB²⁺ (Veronal buffered saline containing 1% BSA, 0.5 mM MgCl₂, 2 mM CaCl₂, and 0.05% Tween 20) for 1 h at 37°C. Deposition of C3 was detected by Dig-conjugated rabbit anti-mouse C3 followed by incubation with anti-DIG-HRP (Boehringer Mannheim). Finally, ABTS (Sigma-Aldrich) with H₂O₂ was added as a substrate for HRP and OD was assessed at 415 nm.

Statistical analysis

Statistical analysis was performed using Student's *t* test for unpaired samples. Values of *p* < 0.05 were considered statistically significant. Data are expressed as mean \pm SEM.

Results

KpOmpA induces an inflammatory response in the air pouch model

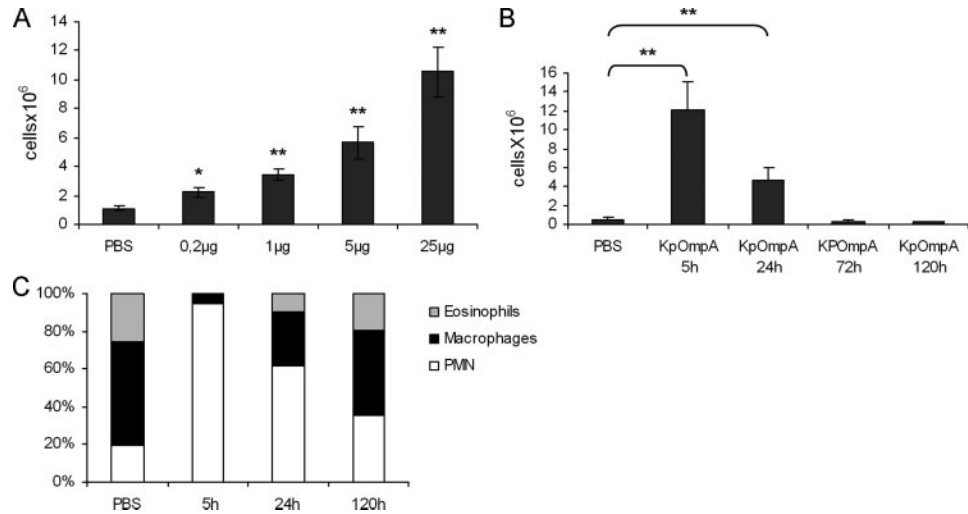
In the air pouch model, a mechanical disruption of the s.c. connective tissue is achieved by repeated injection of air and the resulting cavity develops a structure with features of synovial lining. This model is used to study leukocyte recruitment in the air pouch after injection of an inflammatory stimulus (24). To characterize the model with KpOmpA as an inflammatory stimulus, KpOmpA was given to the mice at different doses (0.2, 1, 5, and 25 μ g/mouse) and pouch lavage was performed 5 h post injection. As shown in Fig. 1A, KpOmpA induced dose-dependent leukocyte recruitment.

To determine the kinetic of leukocyte recruitment and the cellular composition of the infiltrate, mice were sacrificed at different time point (5, 24, 72, and 120 h) after the injection of the stimulus (Fig 1, B and C). KpOmpA induced leukocyte recruitment with a typical kinetic: granulocytes were the first cells to be recruited representing 84% at 5 h, whereas their percentage decreased to 61 and 35% at 24 and 120 h after injection, respectively. Macrophages were recruited with a slower kinetic, being 5% of recruited leukocytes at the early time point and the 28 and 45% at 24 and 120 h, respectively (Fig. 1C). Therefore, KpOmpA is a proinflammatory stimulus that, in the air pouch model in 129/Sv mice, induces a typical inflammatory response characterized by an initial neutrophil recruitment followed by macrophage arrival, which is still persisting 24 h after injection and decreases in 72 h (Fig. 1B).

PTX3 amplifies the inflammatory response to KpOmpA

PTX3 was recently shown to bind KpOmpA in a calcium-dependent manner and to be involved in inflammatory responses induced *in vivo* by OmpA by an unexplained mechanism of amplification of inflammation (14). To investigate the *in vivo* role played by PTX3 in the inflammatory response induced by KpOmpA, PTX3 was coinjected with KpOmpA in the air pouch model. To allow the formation of PTX3-KpOmpA complexes, the two molecules were preincubated for 30 min in Ca²⁺/Mg²⁺ PBS before injection. In the experiments reported in Fig. 2A, representative of 12 performed, mice were injected in the pouch with 25 μ g of PTX3, 25 μ g of KpOmpA, or the combination and pouch lavage was performed 5 h post injection. PTX3 alone did not induce leukocyte recruitment, compared with the vehicle ($0.48 \pm 0.061 \times 10^6$ cells and $0.82 \pm 0.17 \times 10^6$ cells, for PTX3 and PBS, respectively) confirming previous results suggesting that this molecule alone does not cause inflammatory responses. KpOmpA elicited the recruitment of $4.59 \pm 0.67 \times 10^6$ cells in 5 h, whereas the combination of

FIGURE 1. KpOmpA induces an inflammatory response in vivo in the air pouch model. **A**, Dose-response curve following KpOmpA injection. Leukocyte recruitment was assessed 5 h after KpOmpA injection. Results are mean \pm SEM ($n = 5$). **B**, Kinetic of KpOmpA elicited leukocyte recruitment. Pouch lavage was performed at different time points following injection with 5 μ g of KpOmpA. **C**, Cell count performed on cytospin slides stained with Diff-Quick. Results represent the relative percentage of recruited leukocytes. PMN, Polymorphonuclear leukocytes. *, $p \leq 0.05$; **, $p < 0.01$, Student's t test.



KpOmpA and PTX3 induced a total cell recruitment of $10.22 \pm 3.67 \times 10^6$ cells ($p < 0.05$).

To study the dose dependent effect of PTX3, mice were injected with 5 μ g of KpOmpA associated with scalar doses of PTX3 (1, 5, and 25 μ g). As shown in Fig. 2B, the minimal effective dose of PTX3 was 25 μ g ($p < 0.01$).

To assess whether PTX3 modified the cellular composition of the infiltrate, differential counts were performed. In both experimental groups, injected with KpOmpA alone (25 μ g) or with KpOmpA and PTX3 (25 μ g), neutrophils represented 80% of recruited cells 5 h after the injection. These results suggest that PTX3 did not

qualitatively modify the inflammatory response induced by KpOmpA (data not shown).

To further characterize the inflammatory amplification induced by PTX3, we measured two primary inflammatory mediators, IL-6 and JE/CCL2, in air pouch exudates. As shown in Fig. 2, C and D, representative of three experiments performed, the levels of both IL-6 and JE were four- and three-fold higher, respectively, in the exudates from mice treated with the combination of PTX3 and KpOmpA (IL-6, 0.93 ± 0.28 ng/ml; JE, 1.20 ± 0.3 ng/ml) compared with the levels found in mice treated with KpOmpA alone (IL-6, 0.19 ± 0.05 ng/ml; JE, 0.39 ± 0.06 ng/ml; $p < 0.05$).

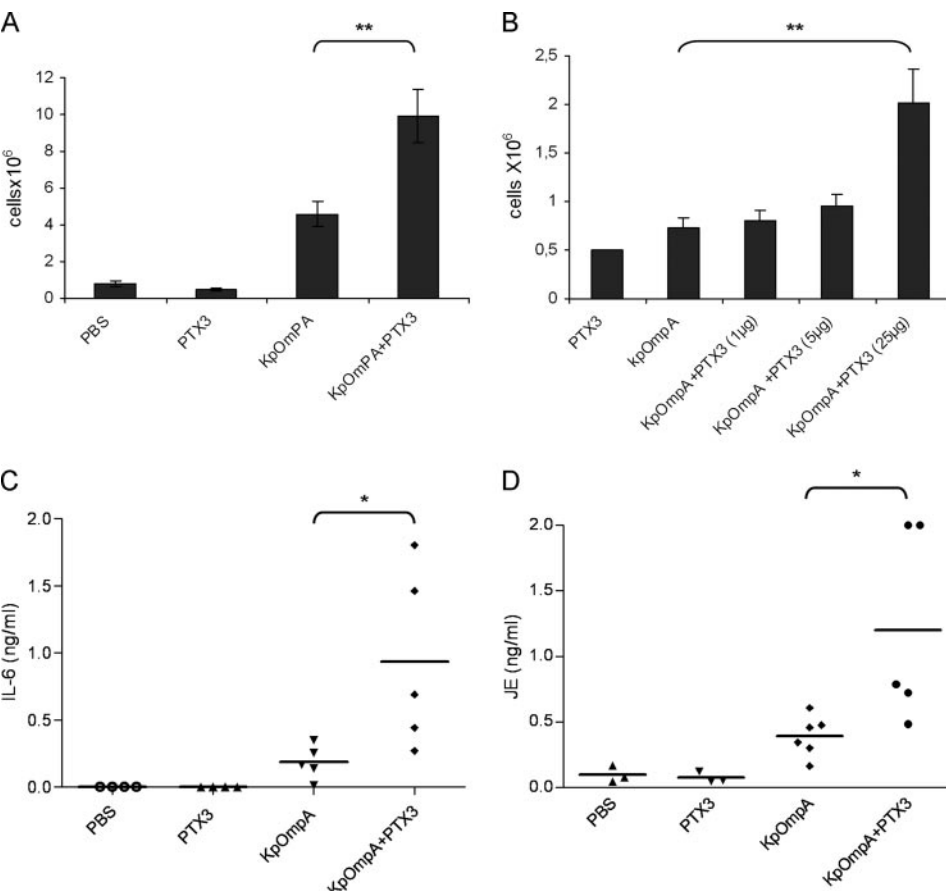


FIGURE 2. Increased inflammatory response in the air pouch from mice treated with the association of KpOmpA and PTX3. **A**, PTX3 amplifies the inflammatory response to KpOmpA. Mice received s.c. dorsal injection of either 25 μ g KpOmpA, 25 μ g PTX3, or the combination of both. Air pouches were washed 5 h post injection of the stimulus and total cells count was performed. Results are mean \pm SEM. Data shown are representative of 12 independent experiments. **B**, Mice were injected in the air pouch with 5 μ g of KpOmpA and scalar doses of PTX3 and cells count was performed 5 h post injection. Results are mean \pm SEM. **C** and **D**, Levels of IL-6 and JE in the air pouch lavage fluids. Results are mean \pm SEM and representative of three independent experiment. *, $p \leq 0.05$; **, $p < 0.01$, Student's t test.

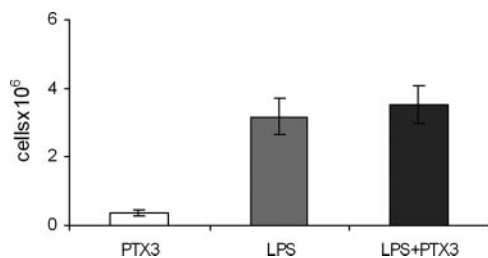


FIGURE 3. PTX3 does not modify the inflammatory response induced by LPS, a nonrecognized microbial moiety. Mice ($n = 6$) were treated with LPS (200 ng) or the association of LPS and PTX3 (25 μ g) and after 5 h pouches were washed as described and total cell count performed.

We next assessed whether the KpOmpA-induced inflammatory response in the air pouch model was influenced by endogenous PTX3. In the four experiments performed with 129/Sv, wild-type and *ptx3*-deficient mice, cell recruitment as well as IL-6 and JE induced by KpOmpA were comparable in the two experimental groups. Consistently with these results, Northern blot analysis of the air pouch lining tissue in wild-type mice treated with KpOmpA revealed a very low induction of PTX3 mRNA, and an ELISA indicated that the protein in the exudates was below the detectable level (data not shown). These results suggest that differently from other inflammatory models in which endogenous PTX3 induced by KpOmpA played an amplification role (14), in the air pouch model, endogenous production of PTX3 is too low to amplify the response.

PTX3 mediated amplification of the inflammatory responses is specific for recognized microbial moieties

To assess the specificity of PTX3-mediated effects in inflammation induced by microbial moieties, mice were challenged with LPS, which is not a PTX3 ligand, and the combination of LPS and PTX3 in the air pouch. As shown in Fig. 3, the combination of LPS and PTX3 did not lead to enhanced inflammatory cell recruitment compared with administration of LPS alone ($3.54 \pm 0.54 \times 10^6$ cells and $3.18 \pm 0.54 \times 10^6$ cells, respectively).

A similar result was observed when IL-1 was injected in the air pouch as inflammatory stimulus alone or in combination with PTX3 (data not shown).

Thus, PTX3 is a humoral mediator which amplifies the inflammatory response elicited by a recognized microbial moiety and it

does not cause a generalized increase of the inflammatory response to proinflammatory stimuli.

PTX3-induced amplification of KpOmpA-mediated inflammation is not mediated by leukotrienes and prostaglandins

Leukotrienes and prostaglandins are classical mediators of the inflammatory response being chemotactic factors for leukocytes in several models of inflammation (25). In an effort to investigate the mechanism responsible for the enhanced inflammatory response mediated by PTX3, we assessed the role of these lipidic mediators in the KpOmpA-mediated inflammation and in PTX3-mediated amplification loop, by treating mice with the 5-lipoxygenase inhibitor MK 886 or with the cyclooxygenase inhibitor indomethacin 1 h before the stimulation with KpOmpA and PTX3. As shown in Fig. 4, leukocyte recruitment induced by KpOmpA was significantly affected by the administration of the inhibitors MK 886, indomethacin and their combination, being reduced to ~30–40% of the response in the absence of inhibitors, suggesting the participation of these lipid factors in KpOmpA-induced inflammation. By contrast, the amplification of leukocyte recruitment induced by PTX3 was not impaired when mice were treated with the inhibitors, as the response remained significantly higher ($p < 0.05$ – $p < 0.0001$) than the response observed in the absence of PTX3. These results indicate that lipid mediators (leukotrienes and prostaglandins) are not involved in mediating the PTX3-induced amplification of inflammation.

PTX3 induced amplification is mediated by complement activation

As PTX3 binds to C1q modulating the activation of the complement cascade, we next studied the role of complement in this model. In a first set of experiments, mice were depleted of complement by CVF injection 24 h before administration of the inflammatory stimulus. CVF is a structural and functional analog of the complement component C3 which leads to the formation of a stable C3/C5 convertase (the complex CVF, Bb), that continuously hydrolyzes C3 and C5, ultimately resulting in complement depletion (26).

As reported in Fig. 5A, which shows one of three experiments performed, depletion of complement by CVF did not affect (or marginally reduced in other two experiments; data not shown) the KpOmpA-induced leukocyte recruitment ($2.57 \pm 0.4 \times 10^6$ cells and $2.11 \pm 0.60 \times 10^6$ cells in the absence and presence of CVF treatment, respectively). By contrast, the treatment significantly

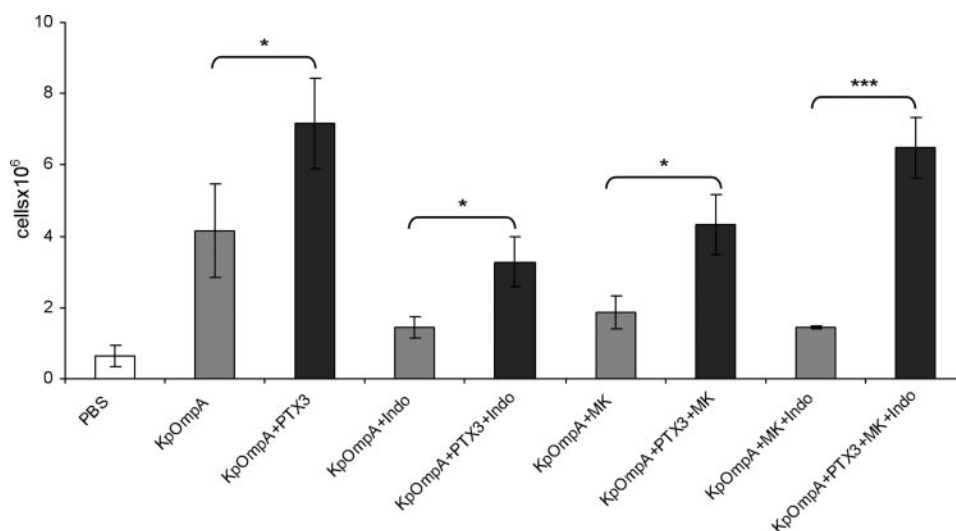
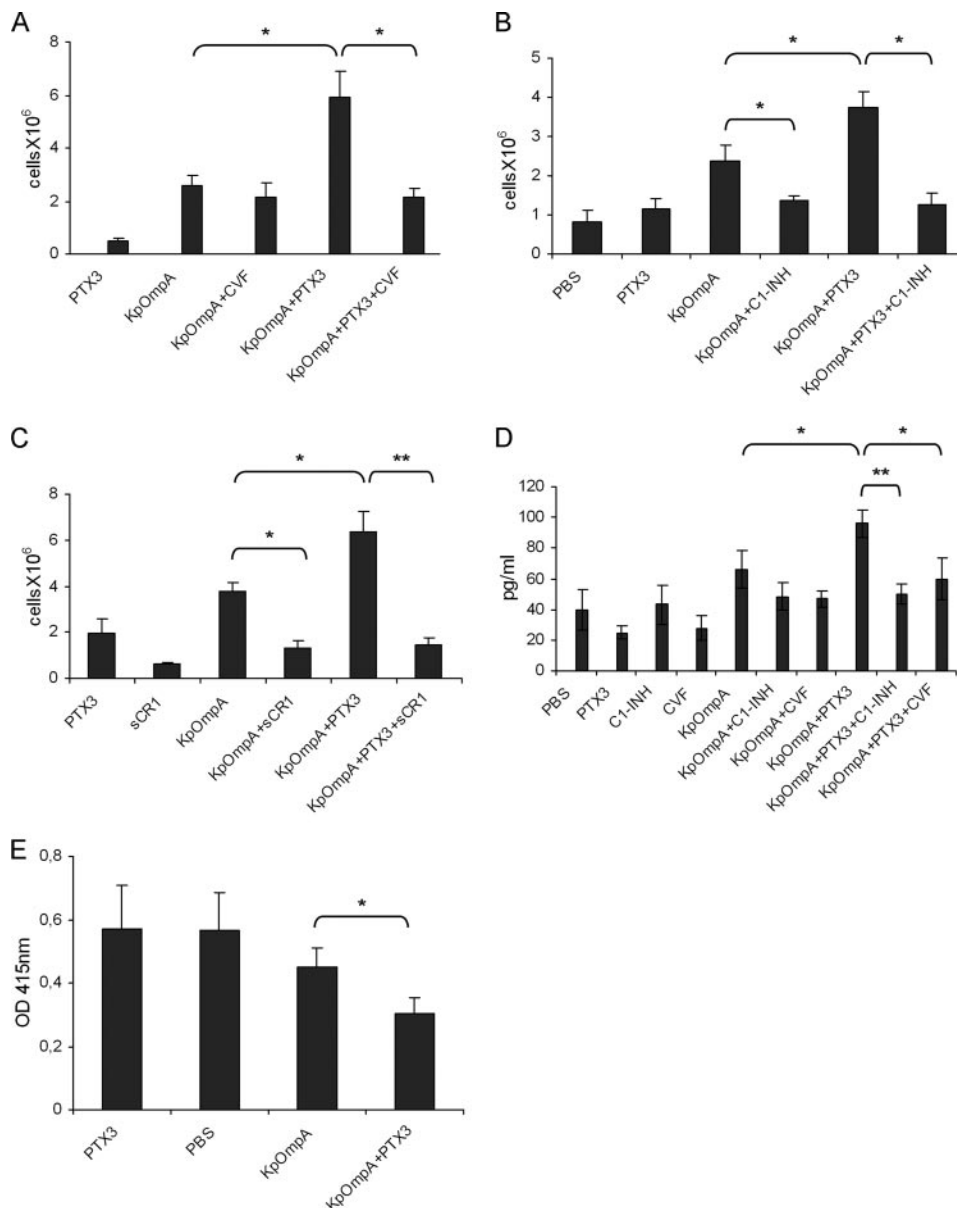


FIGURE 4. Effect of MK 886 and indomethacin on KpOmpA-induced leukocyte recruitment. Mice were first treated with MK886 (MK) and indomethacin (indo) 1 h before receiving 25 μ g KpOmpA, 25 μ g PTX3, or the combination of both. Pouch lavage was performed 5 h post injection and total cells counted. Results are mean \pm SEM ($n = 4$). *, $p \leq 0.05$; ***, $p = 0.0001$, Student's t test.

FIGURE 5. PTX3 induced amplification of the inflammatory response to KpOmpA is mediated by complement activation. A–C, Effect of complement depletion by Cvf or inhibition by C1-INH and sCR1 on PTX3 mediated amplification of cell recruitment. Mice were treated with with 5 μ g KpOmpA (A) or 25 μ g KpOmpA (B and C) and 25 μ g PTX3 or their combination. Pouch lavage was performed 5 h post injection. Results are mean \pm SEM ($n = 5$ –6) and representative of two-three experiments. D, Effect of complement depletion/inhibition by Cvf and C1-INH on PTX3 mediated amplification of JE/CCL2 production. JE was measured in the air pouch exudates at 5 h. Results are mean \pm SEM ($n = 5$ –6). E, Effect of PTX3 in C3 consumption induced by KpOmpA in vivo. C3 deposition on IgM coated plates indicates C3 consumption in sera obtained from treated mice at the time of cell collection from the air pouches. *, $p \leq 0.05$; **, $p < 0.01$, Student's t test.



inhibited the PTX3-induced amplification of the inflammatory response to KpOmpA as the number of infiltrating leukocytes was similar to that observed using KpOmpA alone ($2.11 \pm 0.60 \times 10^6$ cells and $2.16 \pm 0.3 \times 10^6$ cells, in the absence and presence of PTX3, respectively).

The contribution of complement was further assessed by treating mice with C1-INH, which inhibits both the classical and lectin pathway of complement (27), 1 h prior administration of OmpA. As shown in Fig. 5B, representative of one of two experiments performed, C1-INH significantly blocked OmpA activity and abolished PTX3-mediated amplification of the inflammatory response in terms of cell recruitment. Similarly, sCR1 treatment, which inhibits C3/C5 convertases of alternative, lectin, and classical pathways (28), abolished PTX3 activity (Fig. 5C). Inhibition of complement with Cvf and C1-INH significantly reduced PTX3-mediated amplification also in terms of cytokine production (Fig. 5D).

C activation by OmpA in comparison with OmpA and PTX3 was examined in vivo by measuring consumption of classical pathways activity up to C3 in sera obtained from mice at the time of cell collection from the air pouches. The results of this experiment

confirm higher complement consumption through the classical pathway in mice treated with OmpA associated with PTX3 compared with mice treated with OmpA alone, as C3 deposition on IgM coated plates was significantly ($p < 0.05$) reduced in the first group (higher consumption) compared with the second one. Results are shown in Fig. 5E.

Collectively, these results suggest that the activation of complement by PTX3 is involved in the amplification loop set in motion by KpOmpA.

Discussion

The long pentraxin PTX3 is a fluid-phase PRR, which plays a nonredundant role in resistance against selected pathogens. PTX3 has properties similar to Abs: its production is induced by pathogen recognition, and it recognizes microbial moieties, activates complement, and facilitates recognition by phagocytes (8, 10, 12). A microbial moiety recognized by PTX3 is OmpA, a major outer membrane protein of Gram-negative *Enterobacteriaceae*. Cellular recognition of KpOmpA is mediated by two members of the scavenger receptor family, LOX-1 and SREC-I, whereas cellular activation by KpOmpA is cooperatively mediated by TLR2 (14, 29).

Activation of cellular innate immunity by KpOmpA is followed by induction of PTX3, which in turn binds KpOmpA with high affinity (14). Defective local inflammation elicited by KpOmpA, observed in *tlr2*- and *ptx3*-deficient mice, supports that both the cellular and the humoral arms of innate immunity are essential for a full response to KpOmpA. The results reported here demonstrate that PTX3 activates a humoral amplification loop in vivo in response to a microbial ligand mediated by complement activation. The proinflammatory role played in vivo by PTX3 in the presence of a microbial moiety is not generalized, but restricted to a recognized ligand.

KpOmpA induces inflammatory responses in vivo which are mediated by TLR2 activation, and to a lower extent, by PTX3. In particular, in the model of footpad swelling, the inflammatory response is abolished in *tlr2*-deficient mice and significantly reduced in *ptx3*-deficient mice. In the air pouch model, KpOmpA induces a dose-dependent inflammatory response characterized by neutrophil recruitment, in the first hours (>80% at 5 h), followed by macrophage recruitment (60% in 72 h). In this model, we did not observe differences in the inflammatory response between wild-type and *ptx3*-deficient mice. This result suggests that in this model of inflammatory response, endogenous PTX3 was not involved. Consistently, *ptx3* mRNA was very weakly expressed in the air pouch walls and PTX3 levels were undetectable in the air pouch exudates. However, when KpOmpA and PTX3 were cocultured prior s.c. injection, in conditions that allow the association of the two molecules, we observed a significant increase of the inflammatory response compared with KpOmpA alone. The inflammatory response was significantly amplified in terms of cell recruitment and levels of inflammatory cytokines (IL-6 and JE), in a dose-dependent manner.

It was important to assess whether PTX3 acts as a humoral amplifier of inflammatory responses in general or whether its role is specific for recognized ligands. The absence of amplification of the response induced by LPS or IL-1 indicates that the humoral amplification loop mediated by PTX3 is activated only upon recognition of the microbial ligand. This result is consistent with previous results in infection models in which PTX3 deficiency did not cause generalized impairment of host resistance to microbial pathogens, thus suggesting that PTX3 is involved in recognition and resistance against specific microorganisms (12, 13, 19).

Previous studies conducted in vitro indicated that PTX3 did not modify the responsiveness of macrophages or dendritic cells to KpOmpA and did not act as a component of signaling receptor complexes (14). Unlike other soluble receptors that present ligands to cellular signaling receptors, as for instance, soluble IL-6 receptor for IL-6 (24), PTX3 is a humoral KpOmpA recognizing molecule which does not interfere with cellular responses induced by KpOmpA. The effect of amplification of the inflammatory response to KpOmpA played by endogenous PTX3 in the footpad swelling or by recombinant PTX3 in the air pouch model, suggested the activation in vivo of an amplification loop of the inflammatory response independent from cellular receptors recognizing this microbial moiety. PTX3 binds to C1q and modulates the activation of the complement cascade (10, 11). Moreover, the opsonizing activity of PTX3 toward conidia and zymosan (12, 13) implies a cellular PTX3 receptor. Actually, a PTX3 binding site is expressed on phagocytes (12). It was, therefore, important to determine the relative importance of these two pathways in the inflammatory amplification loop activated by PTX3. In this study, we assessed the involvement of the complement in the amplification system of the response elicited by KpOmpA associated to PTX3. The experiments with CVF to deplete mice of complement components, with C1-INH, which inhibits both the classical and

lectin pathways of complement (27) or with sCR1, which inhibits C3/C5 convertases of both alternative, lectin, and classical pathways (28), indicated that the complement is actually involved in the inflammatory response elicited by the association of PTX3 and KpOmpA, as after complement inhibition with these three different treatments, the amplification of the response induced by PTX3 was completely abolished. All together these results strongly support the involvement of the activation of the complement cascade as a nonredundant element of the humoral amplification system of the response to KpOmpA.

This study further unravels the complementary roles played by the cellular and soluble arms of innate immunity in the recognition of microbial ligands. The scavenger receptors LOX-1 and SREC-1 are essential for binding to KpOmpA and TLR2 for signaling. The inflammatory program activated by KpOmpA includes the activation of the soluble arm of innate immunity, i.e., induction of PTX3, which in turn binds to KpOmpA leading to the activation of the complement cascade. Thus, PTX3 activates a complement-dependent pathway of amplification of the innate immune response to cognate microbial ligands.

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Disclosures

The authors have no financial conflict of interest.

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