Anti-inflammatory role in septic shock of pituitary adenylate cyclase-activating polypeptide receptor

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Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are two mediators synthesized by immune cells, specially under inflammatory and antigen stimulation conditions. Reports have shown that neuropeptides attenuate the deleterious consequences of septic shock both by down-regulating the production of proinflammatory mediators and by stimulating the production of anti-inflammatory cytokines by activated macrophages. In this study, we used a knockout for the PACAP receptor (PAC1$^{-/-}$) to demonstrate an important protective role for PAC1 receptor in endotoxic shock. Moreover, our results indicate that PAC1 receptor acts *in vivo* as an anti-inflammatory receptor, at least in part, by attenuating lipopolysaccharide (LPS)-induced production of proinflammatory IL-6, which appears to be the main cytokine regulating the expression of the majority of the acute phase protein genes, which are an important deleterious component of septic shock. Besides, our findings point to endogenously produced VIP and PACAP as participants of the natural anti-inflammatory machinery. Because VIP and PACAP are two attractive candidates for the development of therapies against acute and chronic inflammatory diseases, septic shock, and autoimmune diseases, this paper represents a contribution to the understanding of the mechanism of action of these anti-inflammatory agents.

The inflammatory process is vital for the survival of all complex organisms, and it plays an important role in health and disease. Although the inflammatory process is a localized protective response, the sustained production of inflammatory mediators can lead to serious pathological conditions such as endotoxic shock. Septic shock is a systemic response to severe bacterial infections, generally caused by Gram-negative bacterial endotoxins that induce the generation of proinflammatory factors including tumor necrosis factor-α (TNF-α), IL-6, IL-12, IFN-γ, and NO (1, 2). Thus, the search for endogenous modulators that counterbalance the generation of proinflammatory cytokines without severe effects might reveal an alternative therapy for septic shock.

Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) are members of a subfamily that is based on the homology of both ligands and receptors (3, 4) that are synthesized by immune cells (5, 6), especially under inflammatory and antigen stimulation conditions (7, 8). The immunological effects of VIP and PACAP are exerted through binding to the family 2 of G-protein-coupled receptors, VIP receptors types 1 and 2 (VPAC1 and VPAC2, respectively) and PACAP receptor 1 (PAC1), which stimulate primarily the adenylate cyclase system. Eight splice variants of PAC1 have been described, seven variants coupled to the activation of both adenylate cyclase and inositol phosphate/phospholipase C systems and the eighth variant that activates an L-type calcium channel (9–11). Although both peptides elicit a broad spectrum of functions on innate and acquired immunity, their role is essentially anti-inflammatory (6). VIP and PACAP inhibit endotoxin-induced production of proinflammatory factors such as TNFα, IL-6, IL-12, NO, and IFNγ, and stimulate the production of anti-inflammatory cytokines, such as IL-10 (12–16). In fact, both peptides prevent endotoxic shock in a murine experimental model (17). Although our *in vitro* and *in vivo* studies using specific VIP agonists have indicated that the VPAC1 is the main mediator of the VIP and PACAP anti-inflammatory action (18), the role of PAC1 in inflammation remains undefined. The aim of the present study is to evaluate the role played by PAC1 in the anti-inflammatory action of VIP and PACAP by using a mouse defective in PAC1 expression (knockout of PAC1) in a high-endotoxemia murine model, studying both the *in vivo* and *in vitro* effects of VIP and PACAP on the production of the inflammatory cytokines IL-6 and TNFα. This experimental model demonstrates the *in vivo* involvement not only of VIP/PACAP receptor but of VIP and PACAP produced endogenously on the control of inflammation.

Materials and Methods

Experimental Animals. Adult male and female mice deficient in PAC1 (PAC1$^{-/-}$) were obtained by gene targeting as described (19). PAC1$^{-/-}$ mice were compared with wild-type (C57BL/6 $\times$ 129 Sv) counterparts, which serve as controls. Across all of the experimentation, mice were kept in the animal house in a temperature-controlled room with a 12-h light/dark cycle; free access to standard laboratory chow and water was allowed. Procedures involving animals were conducted in compliance with international laws and policies (European Economic Community Council Directives 86/6091).

Experimental Endotoxemia. Adult (8–10 weeks of age) wild-type and PAC1$^{-/-}$ mice were injected i.p. with different amounts (400–1,000 μg) of lipopolysaccharide (LPS; from *Salmonella enteritidis*; Sigma), and survival was monitored over the next 4–7 days. A 5-nmol dose of VIP or PACAP38 (Novabiochem, Laufelfingen, Switzerland) was administered i.p. concurrently with LPS injection. Control animals received only medium. All survival studies were conducted in a blind and random fashion. Mice receiving LPS (1,000 μg) alone or mice injected with LPS concurrently with VIP or PACAP were killed at various time points. Blood was extracted by cardiac puncture, and peritoneal exudate was obtained as described (12, 13). The blood samples were allowed to clot for 1 h at room temperature; serum was obtained and kept frozen until TNF-α and IL-6 measurement by ELISA. The peritoneal suspension was centrifuged for 5 min at 500 $\times$ *g*, and cell-free supernatant was harvested and assayed for cytokine quantitation.

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Abbreviations: VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; PAC1, PACAP receptor; VPAC1, type 1 VIP receptor; VPAC2, type 2 VIP receptor; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor α.

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Table 1. Comparative effect of LPS on mortality and mean survival of wild-type and PAC1$^{-/-}$ mice

LPS dose, μg per mouse	Mouse type	Mortality, %	Mean survival time, h
400	Wild type	0	NA
	PAC1$^{-/-}$	0	NA
600	Wild type	0	NA
	PAC1$^{-/-}$	50	36 ± 8
1,000	Wild type	87.5	70.3 ± 2.6
	PAC1$^{-/-}$	100	39.2 ± 5.9

Mice (15 animals per group) were injected i.p. with different doses of LPS, and survival was monitored over a 96-h period. Data are expressed as mean ± SD. NA, not applicable.

Macrophage Cultures. Macrophages elicited for 4 days with thioglycollate or resident macrophages were obtained by peritoneal lavage by using 4 ml of RPMI medium 1640 (Life Technologies, Grand Island, NY). Peritoneal exudate cells were washed and resuspended in ice-cold medium supplemented with 2% heat-activated FCS (Life Technologies) containing 2-mercaptoethanol, amino acids, penicillin, and streptomycin. Cells were plated in 96-well tissue culture plates (Corning) at 8×10^4 cells per well in a final volume of 0.2 ml in duplicate. After 2 h at 37°C in 5% CO_2, nonadherent cells were removed by repeated washing. At least 96% of the adherent cells were macrophages as judged by morphological and phagocytic criteria and by flow cytometry. Macrophage monolayers were incubated in complete RPMI medium 1640 and stimulated with different concentrations of LPS (*Salmonella minnesota*; Sigma) (from 1 ng/ml to 10 μg/ml) in the presence or absence of VIP or PACAP38 (from 10^{-12} to 10^{-7} M) at 37°C in a humidified incubator with 5% CO_2. Cell-free supernatants were harvested at the designated time points and kept frozen (−20°C) until assayed for IL-6 and TNF-α production by ELISA.

Cytokine Determination by ELISA. The amount of IL-6 and TNF-α present in supernatants was determined by using specific sandwich ELISA essentially as described (12, 13). Briefly, the capture monoclonal anti-murine IL-6 (clone MP5–20F3) and TNF-α (clone MP6-XT22) antibodies (PharMingen) were used to coat microtiter plates (Corning ELISA plates) at 2 μg/ml at 4°C for 16 h. After washing and blocking with PBS containing 3% BSA, culture supernatants were added to each well for 12 h at 4°C. Unbound material was washed off and the biotinylated monoclonal anti-mouse IL-6 (clone MP5–32C11) and TNF-α (clone MP6-XT3) antibodies (PharMingen) were added at 2 μg/ml for 1 h. Bound antibodies were detected by incubation with avidin-peroxidase for 30 min followed by addition of the 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) (ABTS) substrate solution. Absorbances at 405 nm were measured 30 min after the addition of the substrate. A standard curve was constructed for each cytokine by using various dilutions of murine recombinant cytokines (PharMingen) in PBS containing 10% FCS. The assays were specific for murine IL-6 and TNF-α, respectively.

Statistical Analysis. All values are given as means ± SD. Survival curves were analyzed by the Kaplan–Meier method, and the log-rank test was generated to test the homogeneity between treatment groups. Concentrations of serum and peritoneal cytokines in different experimental groups were analyzed for statistical significance by using Student's t test followed by Scheffé's F test, with $P < 0.05$ as the minimum significant level.

Results and Discussion

Response of Wild-Type and PAC1$^{-/-}$ Mice to High-Dose LPS. To analyze the involvement of PAC1 in endotoxemia, mice defective in PAC1 expression (knockout of PAC1, PAC1$^{-/-}$) and wild-type mice (C57BL/6 × 129 Sv) were injected with different doses of LPS (400, 600, and 1,000 μg per mouse) and survival was monitored. Our results indicated that endotoxin-induced mortality of PAC1$^{-/-}$ mice is higher than the one of wild-type mice, which were resistant to a high dose of LPS (Table 1). In addition, an interesting finding is the fact that even for the nonsurvivors, survival time was twice as long in wild-type mice in comparison to PAC1$^{-/-}$ mice (Table 1). Together, these results demonstrate that PAC1 conferred a remarkable resistance to the lethal effects of LPS.

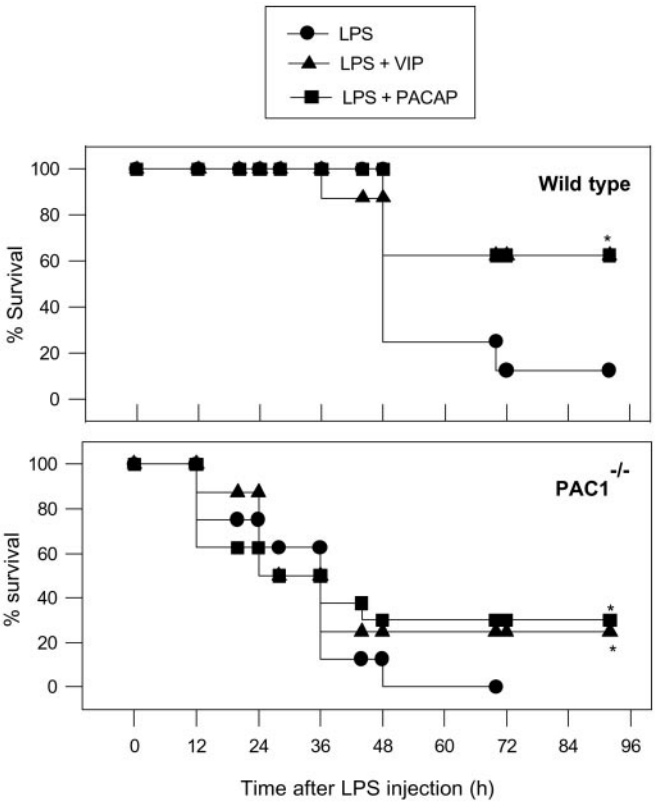

Fig. 1. PAC1 receptor participates in the VIP/PACAP-induced protection from LPS-induced septic shock. Wild-type (C57BL/6 × 129 Sv) (*Upper*) and PAC1$^{-/-}$ (*Lower*) adult mice (12 per group) were injected i.p. with 1 mg of LPS (control) or with LPS plus VIP or PACAP38 (5 nmol). Survival was monitored over the next 5 days, and the survival curves were analyzed by the Kaplan–Meier method. The log-rank test was used for homogeneity between treatment groups (*, $P < 0.001$).

VIP and PACAP have been described as components of the lymphoid microenvironment. We have previously reported that VIP is produced by lymphocytes (5, 7, 20), including the peritoneal population (21). Gaytan *et al.* (22) have found PACAP immunoreactivity in lymphoid organs, and we have demonstrated PACAP expression and synthesis in B and T cell subpopulations (unpublished data). Indeed, several lines of evidence suggest that the production and expression of VIP and of VIP-binding sites are regulated by inflammatory mediators. Recently, we reported that LPS and several proinflammatory cytokines, such as IL-6 and TNF-α, stimulated VIP production in the peritoneal microenvironment (7, 13). Moreover, Kaltreider *et al.* (23) have found an up-regulation of VIP and VIP receptors in a murine model of immune inflammation in lung. Similarly, VIP-binding sites are up-regulated during prolonged strain and energy deficiency after treatment with glucocorticoids on human mononuclear leukocytes, as well as and in casein-

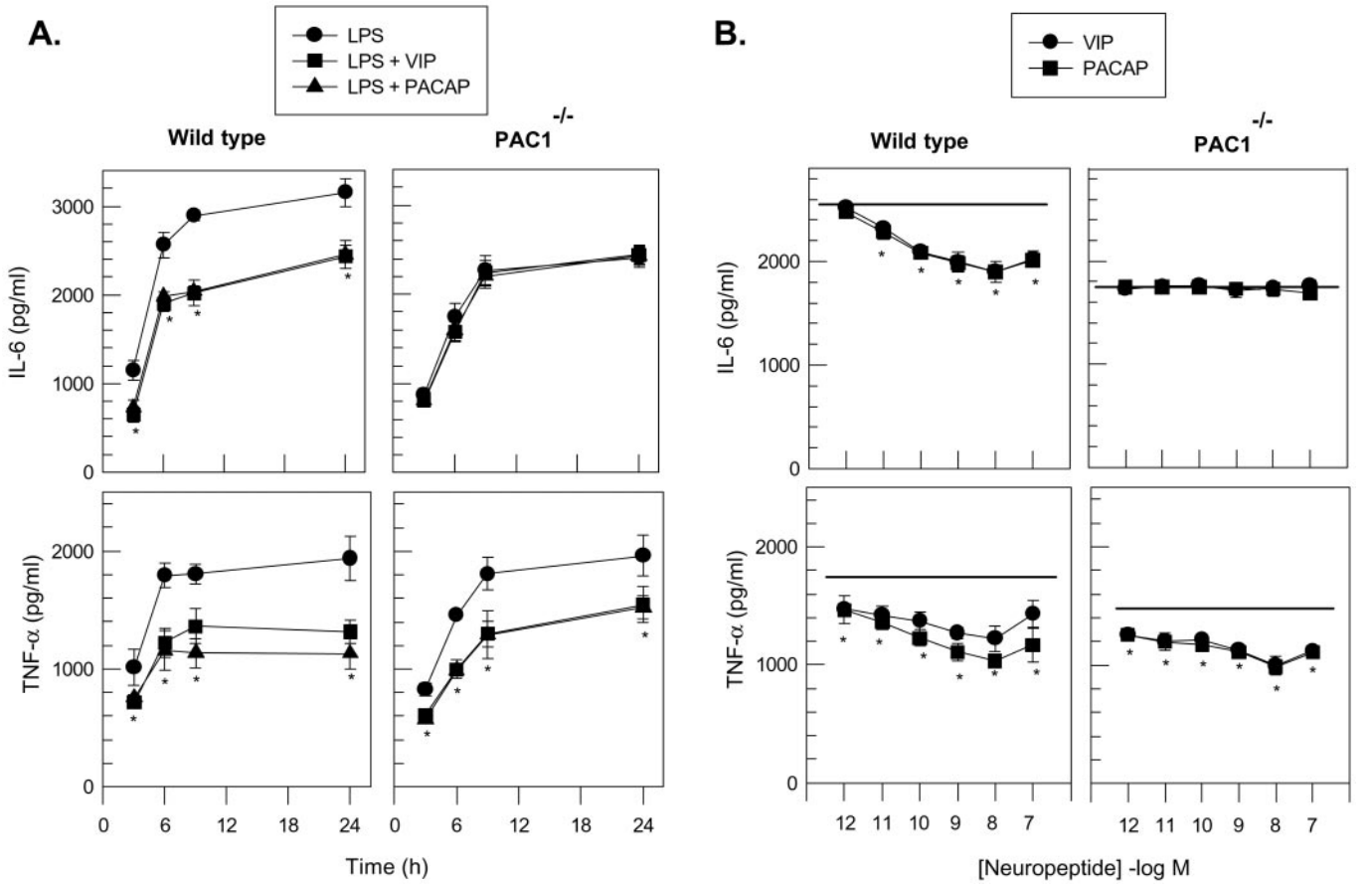

Fig. 2. *In vitro* effect of VIP and PACAP on IL-6 and TNF-α production by PAC1$^{+/+}$ and PAC1$^{-/-}$ macrophages. (*A*) Peritoneal macrophages from wild-type and PAC1-deficient mice were stimulated with 10 ng/ml LPS in absence (control) or presence of 10^{-8} M VIP or PACAP38, and supernatants, harvested at different times, were assayed for cytokine production by ELISA. (*B*) Macrophages from wild-type and PAC1$^{-/-}$ mice were stimulated with 10 ng/ml LPS at a concentration range of VIP or PACAP38 for 6 h, and the contents of IL-6 and TNF-α in the culture supernatants were determined. Each result is the mean ± SD of four separate experiments performed in duplicate. *, $P < 0.001$ with respect to control cultures with LPS alone.

elicited peritoneal macrophages (24). These findings suggest the existence of a negative feedback, in which, after endotoxin stimulation, mediators produced by inflammatory and immune cells induce VIP and VIP/PACAP receptor expression, and their subsequent interactions which negatively regulate the local inflammatory response. In this sense, elevated VIP levels were reported in patients with Gram-negative septic shock and in some endotoxic animal models (25, 26). In addition, an anti-shock effect of PACAP on experimental endotoxin shock in dogs has been reported (27). To our knowledge, our results with PAC1$^{-/-}$ mice may constitute the first demonstration that endogenous VIP and/or PACAP act as true natural anti-inflammatory factors, as well as suggest that signaling through the PAC1 receptor in macrophages is involved in the blockade of the inflammatory responses.

PAC1 Participates in the VIP/PACAP-Induced Protection from LPS-Induced Septic Shock. To investigate the role of PAC1 receptor in the VIP and PACAP protection of lethal endotoxemia, PAC1$^{+/+}$ and PAC1$^{-/-}$ mice were simultaneously injected i.p. with a lethal dose of LPS and VIP or PACAP38, and survival was monitored and analyzed by the Kaplan–Meier method. In wild-type mice, both VIP and PACAP38 protected against the lethal effect of LPS with a survival rate of 60% (Fig. 1 *Upper*), this result is in agreement with our report in BALB/c mice (17). However, the injection of either peptide into PAC1$^{-/-}$ mice prevented death only around 25% of the time (Fig. 1 *Lower*). In a report using selective agonists, we have demonstrated that VPAC1 and VPAC2 receptors are involved in the VIP-mediated anti-inflammatory function, the VPAC1 receptor being the major mediator in the protection from septic shock. However, some data suggest a clear participation of PAC1 in septic shock. In this sense, in agreement with the present study, the PAC1 agonist maxadilan protects against the lethal effect of LPS with a survival rate of ≈25–30% (unpublished data). These data indicate that the PAC1 is physiologically important in the protective action of VIP and PACAP on lethal endotoxemia.

IL-6 and TNF-α Are Differentially Regulated *in Vitro* by VIP/PACAP in Wild-Type and PAC1$^{-/-}$ Mice. Inflammatory factors produced by macrophages are critically involved in the pathogenesis of endotoxemia (1, 2, 28), being the only immunocompetent cells that constitutively express PAC1 (14, 29). We have demonstrated that macrophages are the major targets for the anti-inflammatory effects of VIP and PACAP in our other reports (reviewed in ref. 30). Moreover, it has been demonstrated that, among the list of proinflammatory cytokines involved in endotoxic shock, TNF-α and IL-6 appear to play a pivotal role.

Therefore, to study the involvement of PAC1 on macrophage activation, thioglycollate-elicited peritoneal macrophages from wild-type and PAC1$^{-/-}$ mice were stimulated with LPS in the absence or presence of various doses of either VIP or PACAP38, and the amounts of TNF-α and IL-6 released in the supernatants were assayed by ELISA. Both peptides inhibited in a time-

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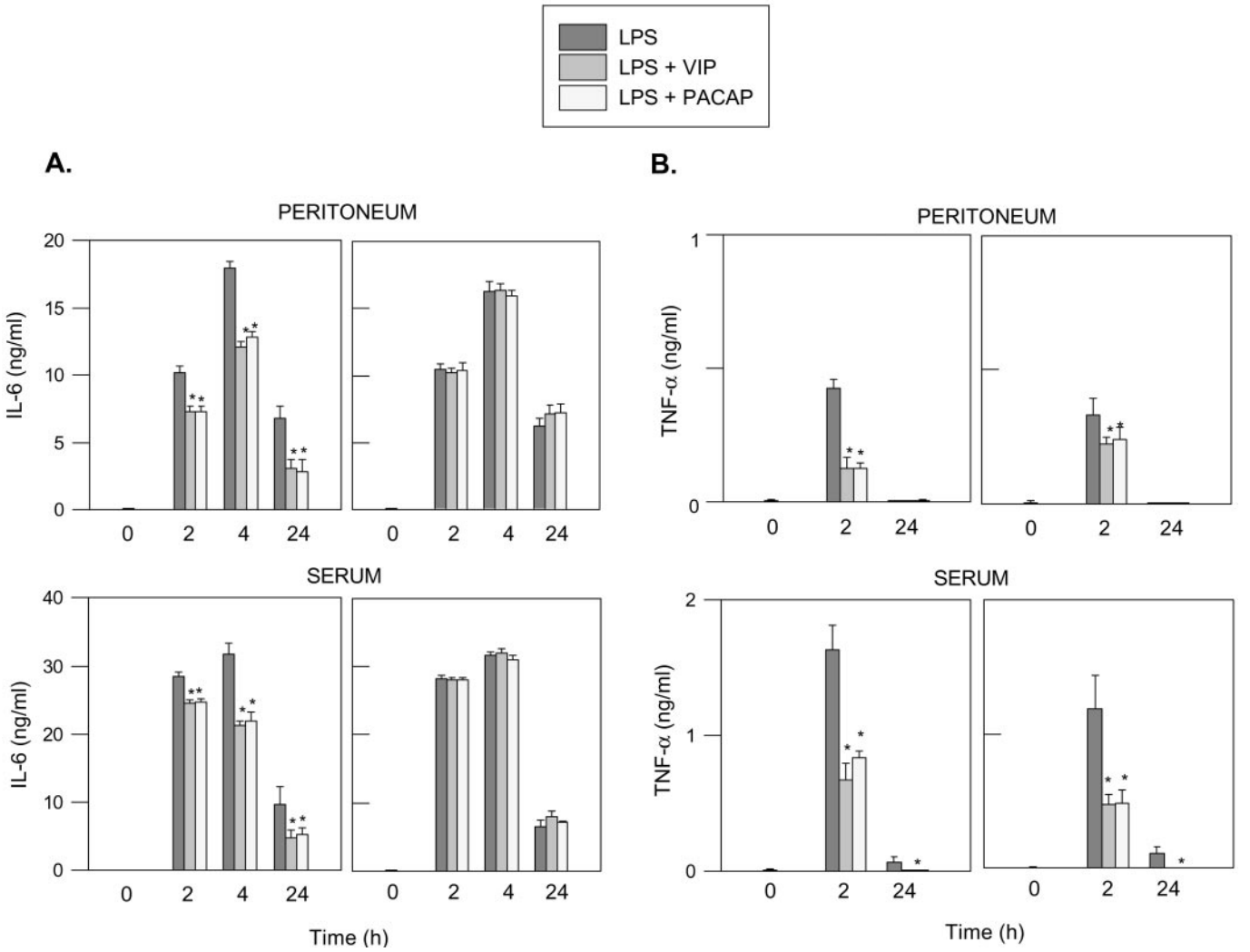

Fig. 3. *In vivo* involvement of PAC1 in endotoxin-induced IL-6 and TNF-α production. Wild-type and PAC1$^{-/-}$ mice were injected i.p. with 1 mg of LPS or LPS plus VIP or PACAP38 (5 nmol per mouse). Groups of six animals were used for each time point. Serum and peritoneal fluid were obtained at the indicated time points and assayed for IL-6 (*A*) and TNF-α (*B*) production by ELISA. *, $P < 0.001$ with respect to control mice without the VIP/PACAP treatment.

dependent manner IL-6 and TNF-α production in wild-type mice (Fig. 2*A*). The time curves indicate that the release of both cytokines was significantly inhibited by VIP and PACAP as early as 3 h, with the maximum inhibitory effect after 6 h of culture ($P < 0.001$; Fig. 2*A*). To determine whether the effect of VIP and PACAP was maintained over a longer period, macrophages were stimulated with LPS in the presence of these peptides during a 24-h incubation period. The reduction of IL-6 and TNF-α release was maintained throughout the 24-h incubation period, indicating that VIP and PACAP do not delay but attenuate IL-6 and TNF-α release, and neither VIP nor PACAP merely alters the time course of IL-6 and TNF-α production by macrophages stimulated with LPS (Fig. 2*A*). The dose–response curves for the two cytokines were similar for VIP and PACAP (Fig. 2*B*), showing maximal effects at 10^{-8} to 10^{-9} M. The inhibitory effect depended on the LPS concentration used (data not shown). For 10 ng/ml LPS, the inhibitory activity of VIP and PACAP reached values of 30% and 35% for IL-6 and TNF-α, respectively (Fig. 2*A*).

In contrast, although VIP/PACAP significantly diminished endotoxin-induced TNF-α levels from PAC1$^{-/-}$ mice in a dose-dependent manner at all times assayed, both peptides failed to inhibit IL-6 production (Fig. 2). These results confirm our findings demonstrating that VIP/PACAP-mediated inhibition of LPS-induced IL-6 production is mainly mediated through binding to PAC1 receptor in macrophages (12), whereas VPAC1 and VPAC2 receptors were ineffective in inhibiting IL-6 release by macrophages (18). Although PAC1 is the selective receptor that binds PACAP with greater affinity than VIP (4), especially in the central nervous system, peritoneal macrophages exhibit only a slight preference for PACAP38 over VIP in the immune system (29), suggesting the existence of a previously uncharacterized subtype of VIP/PACAP receptor.

Regarding TNF-α production, our studies using VPAC agonists in peritoneal and Raw 264.7 macrophages suggest that VPAC1 receptor is the major mediator of the VIP/PACAP inhibitory effect on TNF-α (18, 31). Moreover, our finding has been also supported by the fact that a VPAC1 receptor antagonist, but not PACAP(6–38), an antagonist specific for PAC1 and in a lesser degree for VPAC2, reverses the inhibitory effect of VIP/PACAP on TNF-α production (31). Conversely, Soares *et al.* (32) have described in peritoneal macrophages that pretreatment with maxadilan 2 h before LPS addition decreased LPS-induced TNF-α secretion and enhanced IL-6 production through interaction with PAC1. In previous reports, we have demonstrated that VIP and PACAP exhibit a stimulatory effect

on IL-6 production in both unstimulated and stimulated with very low LPS concentration macrophages, as well as in pretreated macrophages with both peptides 1 or 2 h before LPS addition (33). However, this action was exerted primarily through VPAC1 and partially through PAC1. These discrepancies about the receptor involved in their study are unclear, but the results obtained with PAC1-deficient mice support and confirm previous *in vitro* and *in vivo* studies using agonists and antagonists, indicating that the inhibition of VIP/PACAP on endotoxin-induced IL-6 production is mainly mediated by PAC1, whereas both peptides inhibit TNF-α release in a PAC1-independent way.

PAC1 Receptor Is Involved in the *in Vivo* Inhibition of IL-6 but Not TNF-α Production by VIP/PACAP. Next, an attempt was made to reproduce the *in vitro* observations *in vivo*, treating wild-type and PAC1$^{-/-}$ mice i.p. with LPS alone (1 mg per mouse) or LPS plus VIP or PACAP38 (5 nmol per mouse). Plasma samples and peritoneal suspensions were obtained at 0, 2, 4, and 24 h after injection, and IL-6 and TNF-α levels were evaluated by ELISA. IL-6 levels increased slowly and remained elevated long after LPS injection in both mice, with a peak at 4 h (Fig. 3*A*). In wild-type mice, VIP/PACAP treatment resulted in a inhibition of 30% on IL-6 levels from plasma and peritoneal fluid (Fig. 3*A*). However, the production of this cytokine was not affected by either peptide in PAC1$^{-/-}$ mice (Fig. 3*A*). Regarding TNF-α, a sharp increase in the TNF-α levels was observed within 2 h of the LPS injection in both mouse types (Fig. 3*B*). The addition of VIP or PACAP resulted in a reduction of the levels of secreted TNF-α in serum and peritoneal fluid, with maximum values of 60% (Fig. 3*B*). In agreement with these results, we have recently demonstrated that both peptides reduce the *in vivo* production of TNF-α and IL-6 (13, 17); however, agonists for VPAC1 and VPAC2 had little or no effect on IL-6 production (18). In fact, the PAC1 agonist maxadilan decreased, in a similar way to VIP/PACAP, *in vivo* IL-6 levels in the plasma and peritoneal suspension from endotoxic mice, but it did not modify TNF-α levels (unpublished data). As shown in Fig. 3, the higher circulating levels of these cytokines versus peritoneal fluid cytokine amounts may reflect their potent action during inflammation in this model and point out that the effect to IL-6 and TNF-α is not mainly local. These results indicate that the protective effect of PAC1 is mediated through IL-6 modulation. Although a major role for TNF-α has been demonstrated in septic shock and endotoxemia, the best correlation of plasma cytokine levels with mortality from septic shock has been made with IL-6 (34). IL-6 but not TNF-α levels were found to predict a fatal outcome in patients with septic shock (35). Besides, the systemic administration of IL-6 inhibitors has been demonstrated to protect mice and rats against septic shock induced by the injection of Gram-negative bacteria (36). On the other hand, IL-6 promotes inflammatory events through the expansion and activation of T cells and the differentiation of B cells, and, most importantly, IL-6 appears to be the main cytokine regulating the expression of the majority of the acute phase protein genes, whereas TNF-α regulates a different set of genes (37–39). In addition, it has been demonstrated that the IL-6 system plays an unexpected role in local inflammatory reactions by amplifying leukocyte recruitment by increasing the chemokine production and enhancing adhesion molecule expression (40–42).

A recent paper that used this gene knockout mouse model showed that PAC1 deletion results in a decrease of PACAP-induced insulin secretion, and, more interestingly, these mice exhibited an impairment of glucose-induced insulin secretion and a reduction in glucose tolerance (19). In this sense, inflammatory cytokines have metabolic actions that probably contribute to the general adaptation of the organism during infectious or inflammatory stress. Among cytokines, IL-6 is the main circulating cytokine that acts on glucose metabolism in humans. Fogar *et al.* (43) have recently demonstrated in patients with pancreatic cancer that IL-6, which is released in large amounts by the inflamed pancreas, may contribute to the pathogenesis of diabetes. Moreover, recombinant human IL-6 administration in normal volunteers has been shown to induce dose-dependent increases in fasting blood glucose (44).

In conclusion, taken together these findings demonstrate an important protective role for PAC1 in endotoxic shock and indicate that it acts *in vivo* as an anti-inflammatory receptor by attenuating LPS-induced proinflammatory IL-6 cytokine production. Moreover, we do not rule out that other unknown immunological functions are mediated through this receptor during inflammation. Further studies with this animal model are needed to clarify the involvement of PAC1 in homeostasis and disease.

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1. Morrison, D. C. & Ryan, L. (1987) *Annu. Rev. Med.* **38,** 417–432.
2. Karima, R., Matsumoto, S., Higashi, H. & Matsushima, K. (1999) *Mol. Med. Today* **5,** 123–132.
3. Bockaert, J. & Pin, J. P. (1999) *EMBO J.* **18,** 1723–1729.
4. Harmar, A. J., Arimura, A., Gozes, I., Journot, L., Laburthe, M., Pisegna, J. R., Rawlings, S. R., Robberecht, P., Said, S. I., Sreedharan, S. P., *et al.* (1998) *Pharmacol. Rev.* **50,** 265–270.
5. Gomariz, R. P., Lorenzo, M. J., Cacicedo, L., Vicente, A. & Zapata, A. (1990) *Brain Behav. Immun.* **4,** 151–161.
6. Gomariz, R. P., Martinez, M. C., Abad, C., Leceta, J. & Delgado, M. (2001) *Curr. Pharm. Des.* **7,** 89–111.
7. Martinez, C., Delgado, M., Abad, C., Gomariz, R. P., Ganea, D. & Leceta, J. (1999) *J. Neuroimmunol.* **93,** 126–128.
8. Delgado, M. & Ganea, D. (2001) *J. Immunol.* **166,** 2907–2912.
9. Spengler, D., Waeber, C., Pantaloni, C., Holsboer, F., Bockaert, J., Seeburg, P. H. & Journot, L. (1993) *Nature (London)* **365,** 170–175.
10. Pantaloni, C., Brabet, P., Bilanges, B., Dumuis, A., Houssami, S., Spengler, D., Bockaert, J. & Journot, L. (1996) *J. Biol. Chem.* **271,** 22146–22151.
11. Chatterjee, T. K., Sharma, R. V. & Fisher, R. A. (1996) *J. Biol. Chem.* **271,** 33226–33232.
12. Martinez, C., Delgado, M., Pozo, D., Leceta, J., Calvo, J. R., Ganea, D. & Gomariz, R. P. (1998) *J. Leukoc. Biol.* **63,** 591–601.
13. Delgado, M., Pozo, D., Martinez, C., Leceta, J., Calvo, J. R., Ganea, D. & Gomariz, R. P. (1999) *J. Immunol.* **162,** 2358–2367.
14. Delgado, M., Muñoz-Elias, E. J., Gomariz, R. P. & Ganea, D. (1999) *J. Neuroimmnunol.* **96,** 167–181.
15. Delgado, M., Muñoz-Elias, E. J., Gomariz, R. P. & Ganea, D. (1999) *J. Immunol.* **162,** 1707–1716.
16. Delgado, M., Abad, C., Martinez, C., Leceta, J. & Gomariz, R. P. (2001) *Nat. Med.* **7,** 563–568.
17. Delgado, M., Martinez, C., Pozo, D., Calvo, J. R., Leceta, J., Ganea, D. & Gomariz, R. P. (1999) *J. Immunol.* **162,** 1200–1205.
18. Delgado, M., Gomariz, R. P., Martinez, C., Abad, C. & Leceta, J. (2000) *Eur. J. Immunol.* **30,** 3236–3246.
19. Jamen, F., Persson, K., Bertrand, G., Rodriguez-Henche, N., Puech, R., Bockaert, J., Ahren, B. & Brabet, P. (2000) *J. Clin. Invest.* **105,** 1307–1315.
20. Leceta, J., Martinez, C., Delgado, M., Garrido, E. & Gomariz, R. P. (1996) *Adv. Immunol.* **6,** 29–36.
21. Delgado, M., Pozo, D., Martinez, C., Garrido, E., Leceta, J., Calvo, J. R. & Gomariz, R. P. (1996) *Regul. Pept.* **62,** 161–166.
22. Gaytan, F., Martinez-Fuentes, A. J., Gracia-Navarro, F., Vaudry, H. & Aguilar, E. (1994) *Cell Tissue Res.* **276,** 233–237.
23. Kaltreider, H. B., Ichkawa, S., Byrd, P. K., Ingram, D. A., Kishiyama, J. L., Sreedharan, S. P., Warnock, M. L., Beck, J. & Goetzl, E. G. (1997) *Am. J. Respir. Cell. Mol. Biol.* **16,** 133–144.
24. Segura, J. J., Guerrero J. M., Pozo, D. & Calvo, J. R. (1996) *J. Neuroimmunol.* **64,** 1–7.

25. Brandtzaeg, P., Oktedalen, O., Kierulf, P. & Opstad, P. K. (1989) *Regul. Pept.* **24,** 37–44.
26. Broner, C. W., O'Dorisio, M. S., Rosenberg, R. B. & O'Dorisio, T. M. (1995) *Am. J. Med. Sci.* **309,** 267–277.
27. Suzuki, Y., Kasai, K., Lino, I., Takekoshi, M. & Shimoda, S. (1994) *Life Sci.* **54,** 389–395.
28. Van Snick, J. (1990) *Annu. Rev. Immunol.* **8,** 253–278.
29. Pozo, D., Delgado, M., Martinez, C., Gomariz, R. P., Guerrero, J. M. & Calvo J. R. (1997) *Biochim. Biophys. Acta* **1359,** 250–262.
30. Pozo, D., Delgado, M., Martinez, C., Leceta, J., Guerrero, J. M., Gomariz, R. P. & Calvo, J. R. (2000) *Immunol. Today* **21,** 7–11.
31. Delgado, M., Muñoz-Elias, E. J., Kan, Y., Gozes, I., Fridkin, M., Brenneman, D. E., Gomariz, R. P. & Ganea, D. (1998) *J. Biol. Chem.* **273,** 31427–31436.
32. Soares, M. B. P., Titus, R. G., Shoemaker, C. B., David, J. R. & Bozza, M. (1998) *J. Immunol.* **160,** 1811–1816.
33. Martinez, C., Delgado, M., Pozo, D., Leceta, J., Calvo, J. R., Ganea, D. & Gomariz, R. P. (1998) *J. Neuroimmunol.* **85,** 155–167.
34. Casey, L. C., Balk, R. A. & Bone, R. C. (1993) *Ann. Intern. Med.* **119,** 771–778.
35. Fisher, C. J. J., Opal, S. M. & Dhainaut, J. F. (1993) *Crit. Care Med.* **21,** 318–327.
36. Lundblad, R., Ekstrom, P. & Giercksky, K. E. (1995) *Shock* **3,** 210–215.
37. Fey, G. H., Hocke, G. M., Wilson, D. R., Ripperger, J. A., Juan, T. S. J., Cui, M. Z. & Darlington, G. J. (1994) in *Biology and Pathobiology*, ed. Arias, I. M. (Raven, New York), 3rd Ed., pp. 113–143.
38. Koj, A., Gauldie, J. & Baumann, H. (1993) in *Acute Phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications,* eds. Mackiewicz, A., Kushner, I. & Baumann, H. (CRC, London), pp. 276–283.
39. Pannen, B. H. J. & Robotham, J. L. (1995) *New Horizons* **3,** 183–197.
40. Romano, M., Sironi, M., Toniatti, C., Polentarutti, N., Fruscella, P., Ghezzi, P., Faggioni, R., Luini, W., Van Hinsbergh, V., Sozzani, S., *et al.* (1997) *Immunity* **6,** 315–325.
41. Klouche, M., Bhakdi, S., Hemmes, M. & Rose-John, S. (1999) *J. Immunol.* **163,** 4583–4589.
42. Matsumura, M., Banba, N., Motohashi, S. & Hattori, Y. (1999) *Life Sci.* **65,** PL129–PL135.
43. Fogar P., Basso, D., Pasquali, C., Piva, M. G., Brigato, L., De Paoli, M., Galeotti, F., Corsini, A. & Plebani, M. (1998) *Clin. Chim. Acta* **277,** 181–189.
44. Tsigos, C., Papanicolaou, D. A., Kyrou, I., Defensor, R., Mitsiadis, C. S. & Chrousos, G. P. (1998) *J. Clin. Endocrinol. Metab.* **83,** 337–339.