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Resistance to Lipopolysaccharide Mediated by the *Yersinia pestis* V Antigen-Polyhistidine Fusion Peptide: Amplification of Interleukin-10

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We previously showed that injection of homogenous staphylococcal protein A-V antigen fusion peptide into mice delayed allograft rejection and suppressed the major proinflammatory cytokines tumor necrosis factor alpha (TNF-α) and gamma interferon (IFN-γ) associated with generation of protective granulomas. This study was undertaken to determine if V antigen could prevent endotoxic shock, known to be mediated by excessive production of certain proinflammatory cytokines. After treatment with 50 μg of homogeneous V antigen-polyhistidine fusion peptide (V_h), the 50% lethal dose of purified lipopolysaccharide (LPS) in BALB/c mice immediately rose from 63 μg (normal controls) to 318 μg, fell to near baseline (71 μg) in 6 h, and then slowly rose to a maximum of 566 μg at 48 h before again returning to normal. Injected V_h alone (50 μg) promptly induced the anti-inflammatory cytokine interleukin-10 (IL-10) as well as modest levels of TNF-α (an inducer of IL-10) in spleen. Concomitant injection of V_h and an otherwise lethal dose of LPS (200 μg) dramatically decreased levels of TNF-α and IFN-γ in the spleen and peritoneal lavage fluid as compared to values determined for LPS alone. These results would be expected if V antigen directly up-regulated IL-10 that is reported to generally down-regulate proinflammatory cytokines. Mice receiving 200 μg of LPS 48 h after injection of V_h exhibited patterns of cytokine synthesis similar to those observed in endotoxin-tolerant mice, a condition also reported to be mediated by IL-10. These findings suggest that V antigen serves as a virulence factor by amplifying IL-10, thereby repressing proinflammatory cytokines required for expression of cell-mediated immunity.

The low calcium response is defined as the ability of wild-type yersiniae either to grow at 37°C in the presence of Ca^{2+} (~2.5 mM) while down-regulating LcrF-mediated virulence factors or to remain static in Ca^{2+}-deficient media (≥1.0 mM) while up-regulating these determinants (18, 27, 30, 69) (Lcr^+). The latter include certain Yops (10, 23, 49–51, 56, 57) as well as V and W antigens (12), all of which are encoded on a ~70-kb Lcr plasmid. V antigen (LcrV) was initially assumed to be a major virulence factor (13, 15) due to its abundant production at 37°C but not 26°C by all virulent strains (14, 15), its export both in vitro (31) and in vivo (55), and its ability to raise protective antibodies (14, 31). However, preparations used in early studies consisted of crude (14) or partially purified samples (31) possibly containing other VirF-mediated determinants capable of raising protective antibodies. Mutational loss of V antigen, encoded by *lcrV* located within an *lcrGVH-yopBD* operon (7, 44, 46), eliminated the low calcium response, indicating an alternative role as internal regulator (7, 45, 54). This interpretation seemed inconsistent with mediation of immunity to plague by anti-V antigen because gamma globulin is typically excluded from bacterial cytoplasm. Attempts to verify the putative protective role of anti-V antigen were hindered by the penchant of this 37-kDa monomeric peptide to undergo autodegradation to 28- to 36-kDa fragments during purification by classical biochemical methods (11). Nevertheless, rabbit polyclonal antisera raised against these fragments provided mice with significant passive immunity against experimental plague (61).

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To obtain formal proof that this protection was mediated by anti-V antigen, we engineered a staphylococcal protein, A-V antigen fusion peptide (PAV). Rabbit polyclonal anti-PAV provided marked passive immunity which was eliminated by absorption with defined truncated derivatives of V antigen, demonstrating that at least one protective epitope resided internally within the sequence of amino acids 168 to 275 (39). The intravenous 50% lethal dose of Lcr^+ *Yersinia pestis* in mice is ~10 organisms (60); PAV, the V antigen moiety of a glutathione transferase-V antigen fusion peptide, and a hexahistidine-V antigen fusion peptide (V_h) could actively immunize mice against challenge with ~10^4 (42), ~10^6 (32), and ~10^7 (40) Lcr^+ yersiniae, respectively. It is remarkable that mice infected with Lcr^+ cells of *Y. pestis* are unable to express detectable levels of the major inflammatory cytokines gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) until the onset of morbidity (41). Prior to this symptom, which signals spillage of bacteria into the vascular system (56, 60–62), yersiniae proliferate extracellularly within necrotic foci located primarily in the liver and spleen. These lesions increase progressively in size and eventually coalesce, resulting in eventual loss of organ function and death (42, 62) unless the mice initially receive anti-V antigen (42, 62) or are primed with IFN-γ plus TNF-α (41). Both regimens facilitate a typical visceral inflammatory response characterized by expression of endogenous IFN-γ and TNF-α, infiltration of mononuclear phagocytes, and attendant formation of protective granulomas (42, 62). Proof that cytokine suppression by Lcr^+ yersiniae was at least partially mediated by V antigen was obtained by showing that injected PAV down-regulated IFN-γ and TNF-α normally induced by Lcr^- or *lcrV* yersiniae (42), prolonged the survival of these mutants in vivo (42), enabled *Listeria monocytogenes* to cause acute disease (42), and significantly post-

poned mouse skin allograft rejection (38) known to be mediated by IFN-γ and interleukin-2 (IL-2) (3, 17).

V_h tightly bound genetically linked LcrH (40), suggesting that the latter may serve as a chaperone for V antigen. The reported regulatory activity of V antigen may therefore reflect effects caused by the necessary accumulation of free LcrH in nonpolar *lcrV* mutants. Accordingly, the bulk of evidence now available indicates that the initial assumption that V antigen is a monofunctional virulence factor (13) was correct. The purpose of this report is to show that V_h provides marked resistance to BALB/c mice against the lethal effect of lipopolysaccharide (LPS) known to be caused by excess induction of IL-1, IFN-γ, and TNF-α (1, 8, 20, 21, 64, 65, 70). We demonstrate that this protection is associated with amplification of the anti-inflammatory cytokine IL-10. The latter was reported to prevent endotoxic death by virtue of its ability to down-regulate itself plus the proinflammatory cytokines IL-1, IL-2, IL-6, IL-8, IL-12, IFN-γ, TNF-α, and granulocyte-macrophage colony-stimulating factor (25, 28, 34, 37, 47).

MATERIALS AND METHODS

V antigen fusion peptides. Methods for preparing homogenous V antigen fusion peptides have been described in detail. Briefly, PAV and protein A (PA) were produced by cells of *Escherichia coli* BL21 (F^- *ompT lon* r_B^- m_B^-) (Novagen, Madison, Wis.) transformed with pPAV13 (39) and pRIT5 (Pharmacia, Uppsala, Sweden), respectively. After growth in fermentor vessels containing Luria broth plus ampicillin (50 μg/ml), the cells were harvested and lysed. The resulting extracts were then clarified by centrifugation and subjected to chromatography on immunoglobulin G-Sepharose 6FF (Pharmacia) (39). V_h was similarly produced by cells of *E. coli* BL21(DE3) (Novagen) transformed with pVHB62 (40) in M9ZB medium plus ampicillin (50 μg/ml) upon induction with IPTG (isopropyl-β-D-thiogalactopyranoside). After lysis and clarification, the extract was chromatographed first with chelating Sepharose (Pharmacia) charged with Ni^{2+} and then with Ni-nitrilotriacetic acid agarose (Qiagen, Chatsworth, Calif.) to yield V_h, which was immediately stored at −20°C (40).

The levels of contaminating LPS present in purified V antigen fusion peptides were determined with a QCL-1000 *Limulus* amebocyte lysate kit (BioWhittaker, Inc., Walkersville, Md.) as described in directions provided by the manufacturer. All preparations used for experiments were essentially LPS-free (<1 ng/mg); the 50-μg dose of V_h used to protect mice against endotoxic death contained <33 pg of LPS.

Mice. Female BALB/c mice (8 weeks of age, 19 to 21 g) purchased from Charles River Laboratories (Wilmington, Mass.) were used in all experiments. The mice were kept in a room maintained at a constant temperature of 18.5°C and relative humidity of 35%; commercial food and drinking water were provided ad libitum.

LPS. Commercially available LPS prepared from *Salmonella typhimurium* by the hot phenol method (33) was used in all experiments (Sigma Chemical Co., St. Louis, Mo.). The 50% lethal dose of LPS was calculated by the method of Reed and Muench (48) with at least five dilutions of LPS per determination and five mice per dilution. The weight of each mouse was ascertained before injection to ensure administration of a constant dose per kilogram of body weight.

Cytokine assays. Methods for preparing samples for analysis of cytokines have been described previously in detail (41). This procedure involves sacrificing mice in groups of three at intervals by terminal bleeding under anesthesia before preparation of peritoneal lavage fluid (PLF) and removal of the spleen. PLF was obtained by extraction of the peritoneum with 2 ml of 1% (wt/vol) 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS; Sigma) in Hanks balanced salt solution (GIBCO BRL, Gaithersburg, Md.) (70). Individual samples of blood were allowed to clot overnight at 4°C; sera obtained after centrifugation were used in assays. Spleens were homogenized in Hanks balanced salt solution plus 1% CHAPS, and the resulting preparations were clarified by centrifugation. All samples were stored at −70°C prior to assay.

Commercial solid-phase enzyme immunoassay kits utilizing the multiple-antibody sandwich principle were used to determine IL-2, IL-4, IL-6, IFN-γ, and TNF-α (Endogen, Cambridge, Mass.) levels; IL-10 was assayed with a kit purchased from Biosource International (Camarillo, Calif.). Results were expressed as nanograms of cytokine per milligram of spleen, milliliter of PLF, or milliliter of blood.

Miscellaneous. Phosphate-buffered saline (PBS) was used as a negative control in studies of LPS tolerance. Low-endotoxin (<1 ng/mg) bovine serum albumin (BSA; Sigma), PA, PAV, LPS, and V_h either dissolved or appropriately diluted in PBS were also used in these experiments.

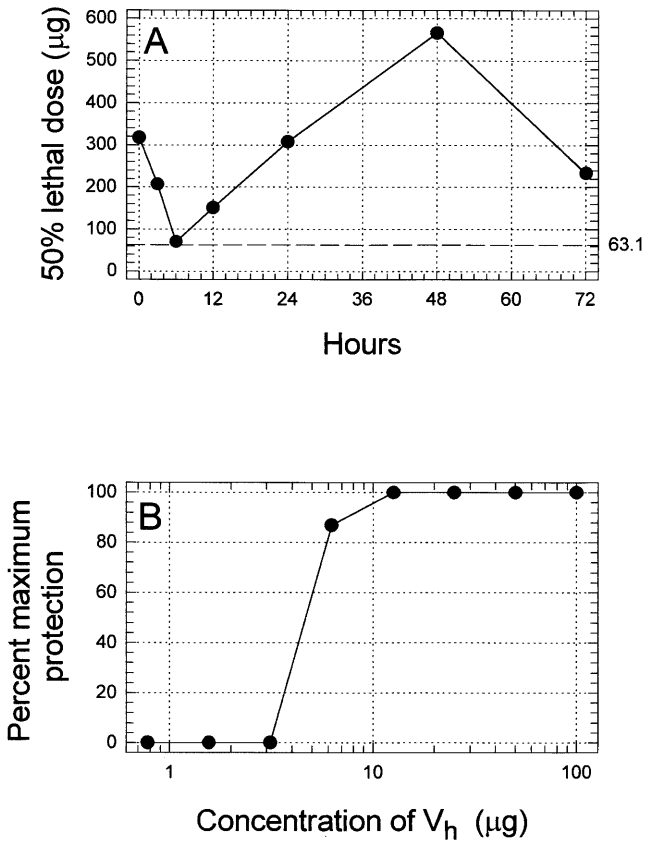

FIG. 1. (A) Fifty percent lethal dose of LPS injected intraperitoneally into BALB/c mice at indicated intervals after intraperitoneal injection of 50 μg of V_h in PBS; the dashed line indicates the base value of 63.1 μg of LPS determined for control mice receiving PBS alone. (B) Percent of maximum protection against lethality of LPS in BALB/c mice determined 48 h after intraperitoneal injection of increasing doses of V_h; the maximum observed 50% lethal dose and base control values are set at 100 and 0%, respectively.

RESULTS

Resistance to LPS. BALB/c mice received 50 μg of homogenous V_h by intraperitoneal injection and were then challenged at intervals thereafter with appropriate concentrations of LPS to permit determination of 50% lethal doses. As shown in Fig. 1A, the 50% lethal dose rose immediately from 63 μg (determined for the same lot of LPS in control mice treated with PBS alone) to 318 μg before falling to near-baseline (71 μg) in 6 h. This value then increased gradually to achieve a maximum of 566 μg by 48 h before again returning to normal. The ability of V_h to increase the 50% lethal dose of LPS was a function of concentration in that maximum resistance occurred in mice receiving ≥12.5 μg although significant protection was obtained with 6.3 but not 3.2 μg (Fig. 1B). A single injection of V_h thus provided dose-dependent immediate resistance to LPS that rapidly subsided only to be replaced by 48 h with a second phase of delayed resistance. V_h (50 μg) boiled for 1 min did not protect against endotoxic death.

Immediate resistance. Mice received V_h (50 μg) alone, a lethal dose (200 μg or three 50% lethal doses) of LPS alone, or both reagents by concomitant intraperitoneal injection and were then sacrificed immediately or after 1.5, 3, 6, or 10 h; the spleen, PLF, and blood were prepared for determination of IL-2, IL-4, IL-6, IL-10, IFN-γ, and TNF-α levels. Patterns of expression in the spleen indicated that immediate resistance to

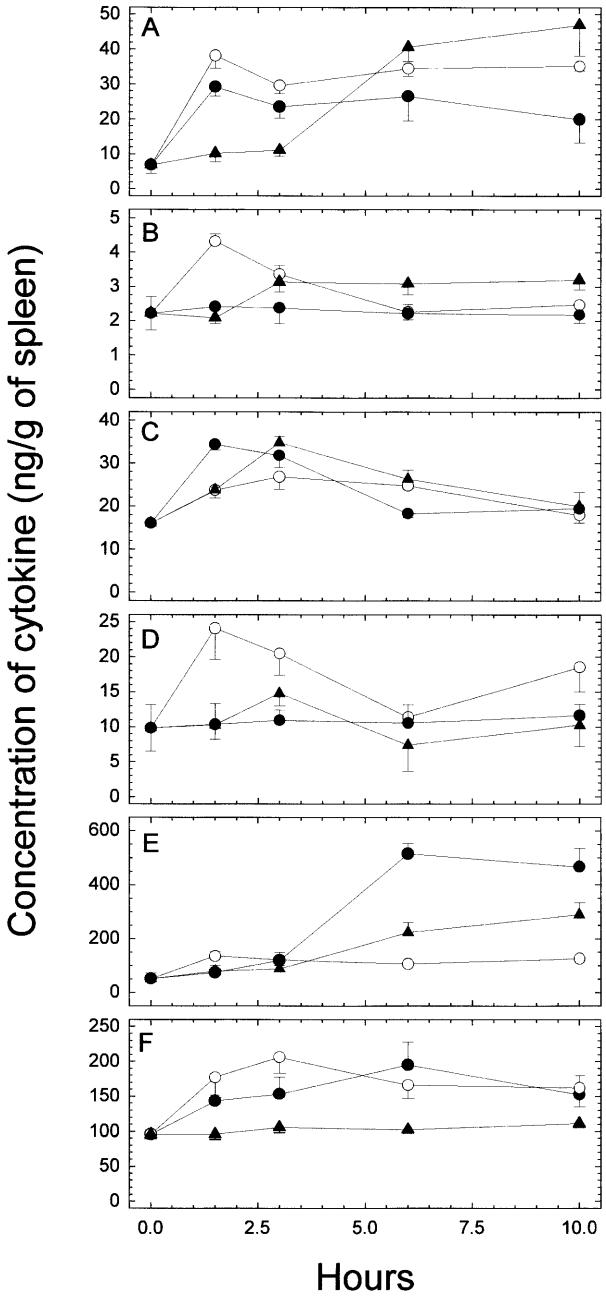

FIG. 2. Concentrations of IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), IFN-γ (E), and TNF-α (F) in spleens of mice receiving an intraperitoneal injection of 50 μg of V_h in 0.1 ml of PBS (○), 200 μg of LPS in 0.1 ml of PBS (●), or 50 μg of V_h plus 200 μg of LPS (▲).

LPS was associated with prompt amplification of the anti-inflammatory cytokines IL-4 (Fig. 2B) and especially IL-10 (Fig. 2D), which underwent an ~2.5-fold increase to achieve a titer approaching 25 ng/g. V_h alone did not up-regulate the important proinflammatory cytokine IFN-γ (Fig. 2E), although significant early synthesis of IL-2 (Fig. 2A) and especially TNF-α (Fig. 2F) was noted. However, titers of IFN-γ and TNF-α were markedly reduced in spleens of mice receiving LPS plus V_h as compared to those receiving LPS alone. We assume that this difference accounts for survival. Levels of all tested cytokines generated in the PLF of mice receiving the lethal dose of LPS were initially higher than those observed after injection of V_h alone or of LPS plus V_h (Fig. 3). Transient appearance of TNF-α in blood after injection of LPS was reduced by concomitant administration of V_h (Fig. 4E), whereas both reagents together enhanced the initial titer of IL-10 over those seen after injection of either V_h or LPS alone (Fig. 4C). Injected V_h also prevented significant LPS-induced accumulation of IFN-γ in blood (Fig. 4D). These results illustrate that early resistance to the lethal effect of LPS mediated by V_h is correlated with modest up-regulation of IL-4, marked

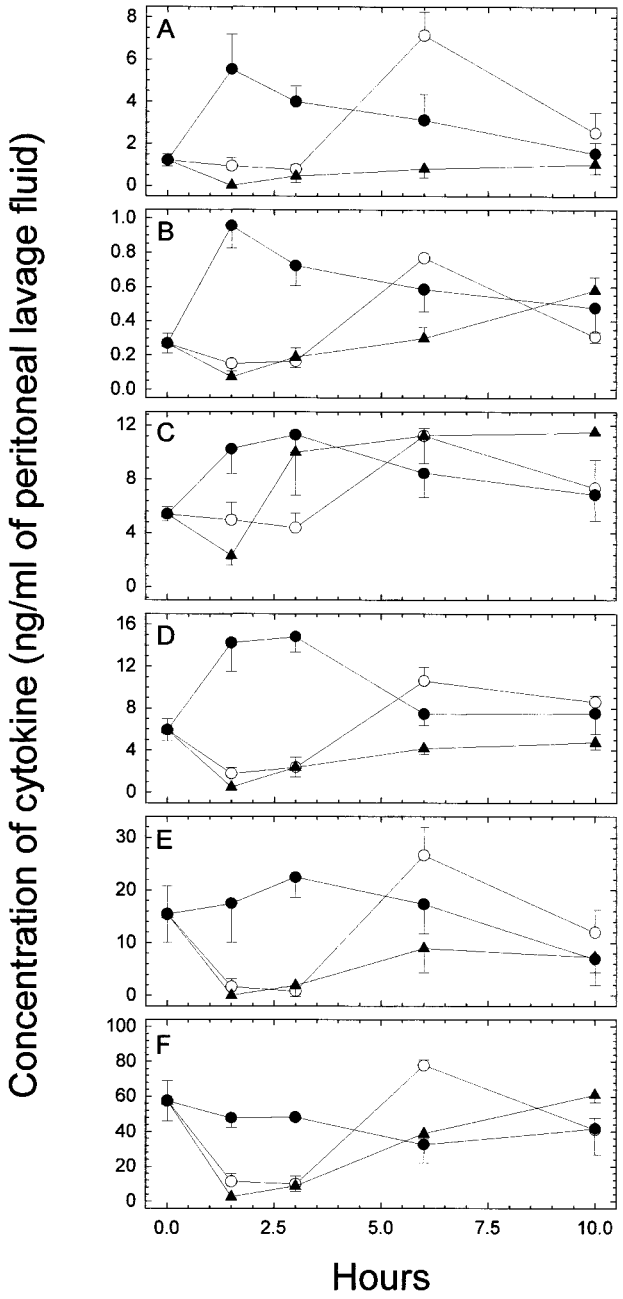

FIG. 3. Concentrations of IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), IFN-γ (E), and TNF-α (F) in the PLF of mice receiving an intraperitoneal injection of 50 μg of V_h in 0.1 ml of PBS (○), 200 μg of LPS in 0.1 ml of PBS (●), or 50 μg of V_h plus 200 μg of LPS (▲).

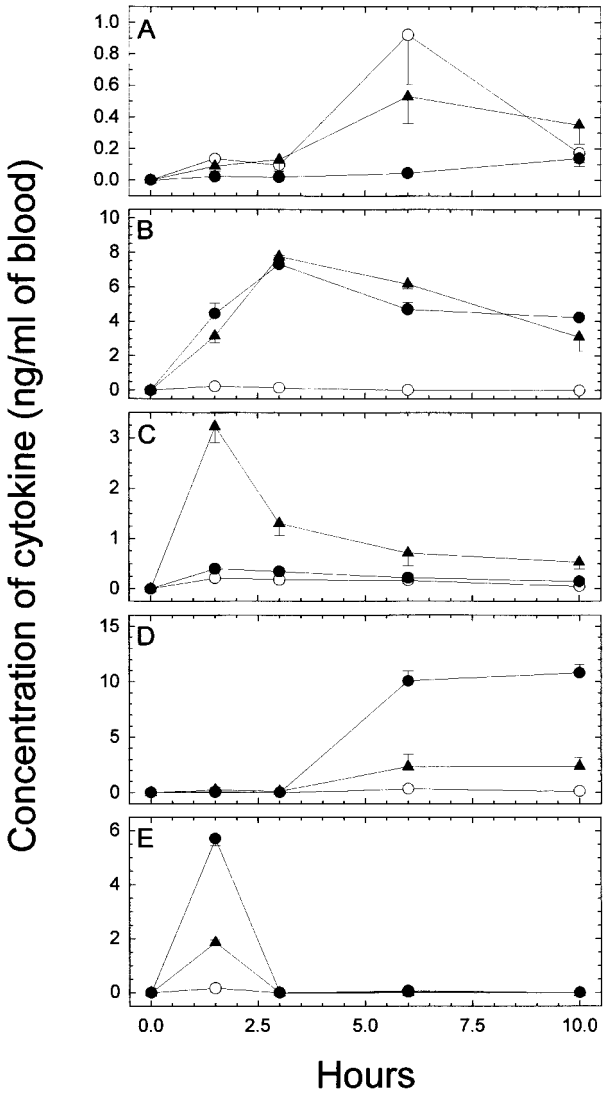

FIG. 4. Concentrations of IL-2 (A), IL-6 (B), IL-10 (C), IFN-γ (D), and TNF-α (E) in blood of mice receiving an intraperitoneal injection of 50 μg of V_h in 0.1 ml of PBS (○), 200 μg of LPS in 0.1 ml of PBS (●), or 50 μg of V_h plus 200 μg of LPS (▲).

up-regulation of IL-10, and general down-regulation of the major proinflammatory cytokines IFN-γ and TNF-α.

Delayed resistance. Mice primed by intraperitoneal injection of PBS, BSA (100 μg), LPS (5 or 25 μg), PA (100 μg), PAV (100 μg), or V_h (50 μg) were challenged 48 h later by intraperitoneal injection of the normally lethal dose of LPS. Priming with LPS, PAV, and V_h, but not PBS, BSA, or PA, provided significant resistance against this challenge (Table 1). In the case of V_h, protection was dependent upon the use of once-thawed reagent; repeated freezing and thawing resulted in loss of protective activity (Table 1). These experiments provided a basis for comparing V_h and LPS as primers affecting expression of cytokines during delayed resistance.

Mice were treated as described (Table 1) with PBS, BSA, LPS, PA, PAV, or V_h and then challenged after 48 h with the normally lethal dose of LPS. Levels of cytokines were then determined after 0, 1.5, 3, 6, and 10 h by the same procedures used to characterize immediate resistance. Results observed after initial injection of PBS and PA closely approximated those illustrated for BSA (Fig. 5 to 7), and concentrations determined upon priming with the 5-μg dose of LPS (Fig. 5 to 7) approached but never exceeded values found after priming with the 25-μg dose (data not shown). Titers assayed in the spleen after treatment with BSA, the 5-μg dose of LPS, and V_h are illustrated in Fig. 5. Levels of cytokines recorded for control mice initially primed with BSA (destined to succumb to endotoxemia in 2 to 3 days) generally exceeded those detected after initial priming with V_h or the sublethal dose of LPS. This effect was especially evident at 6 h for IFN-γ (Fig. 5E) and TNF-α (Fig. 5F). Values obtained for PLF are shown in Fig. 6. Again, priming with V_h or a first round of LPS markedly suppressed the appearance of all six cytokines upon injection of the second round of LPS. This trend was less evident in blood (Fig. 7), where only transient accumulation of TNF-α occurred 1.5 h after challenge with LPS. These findings show that initial treatment of mice with a sublethal dose of LPS (sufficient to promote endotoxin tolerance) or with V_h yields remarkably similar patterns of cytokine expression upon later challenge with an otherwise lethal concentration of LPS. Delayed V_h-mediated resistance to lethality may thus reflect induction of a physiological state mimicking LPS tolerance, a phenomenon also known to be mediated by IL-10 (25, 28, 34, 37, 47, 66, 67).

DISCUSSION

It is established that those proinflammatory cytokines that serve as primary effectors of bacterial resistance by directly or indirectly promoting inflammation and delayed hypersensitivity are primarily produced by either T_H1 cells (e.g., IL-2, IFN-γ, and TNF-β) or professional phagocytes, especially macrophages (e.g., IL-1, IL-6, IL-8, IL-12, and TNF-α). In contrast, those major anti-inflammatory cytokines that typically favor humoral immune responses are primarily expressed by T_H2 cells (e.g., IL-4 and IL-10). Transforming growth factor β can exhibit both capabilities (19). The cytokines produced by each T_H cell subset can often down-regulate those produced by the other subset, thus effectively providing mechanisms which maintain homeostasis (6, 29). Salient examples are up-regulation of IL-10 by TNF-α (63, 68) and general down-regulation of proinflammatory cytokines by IL-4 and, especially, IL-10 (5, 6, 25, 29, 34, 37, 59, 66, 67). The latter processes are typically distinct as judged by the abilities of IL-4 to enhance degradation of proinflammatory cytokine mRNA and of IL-10 to pre-

TABLE 1. Ability of V_h, PAV, and sublethal doses of LPS to protect BALB/c mice against subsequent lethal challenge with LPS

Compound injected	Amt (μg)	No. of thawings	No. of survivors after challenge with 200 μg of LPS[a] at day:							
			0	1	2	3	4	5	6	7
PBS			8	4	0	0	0	0	0	0
BSA	100		8	3	0	0	0	0	0	0
LPS	25		8	8	8	8	8	8	8	8
LPS	5		8	8	8	8	8	8	7	7
PA	100		8	5	2	2	2	2	1	1
PAV	100		8	8	8	7	7	6	6	6
V_h	50	1	8	8	8	8	8	8	8	8
	50	2	4	2	2	2	2	2	2	2
	50	3	4	3	0	0	0	0	0	0

[a] LPS was injected intraperitoneally 48 h after administration of PBS, BSA, LPS, PA, PAV, or V_h at the indicated doses.

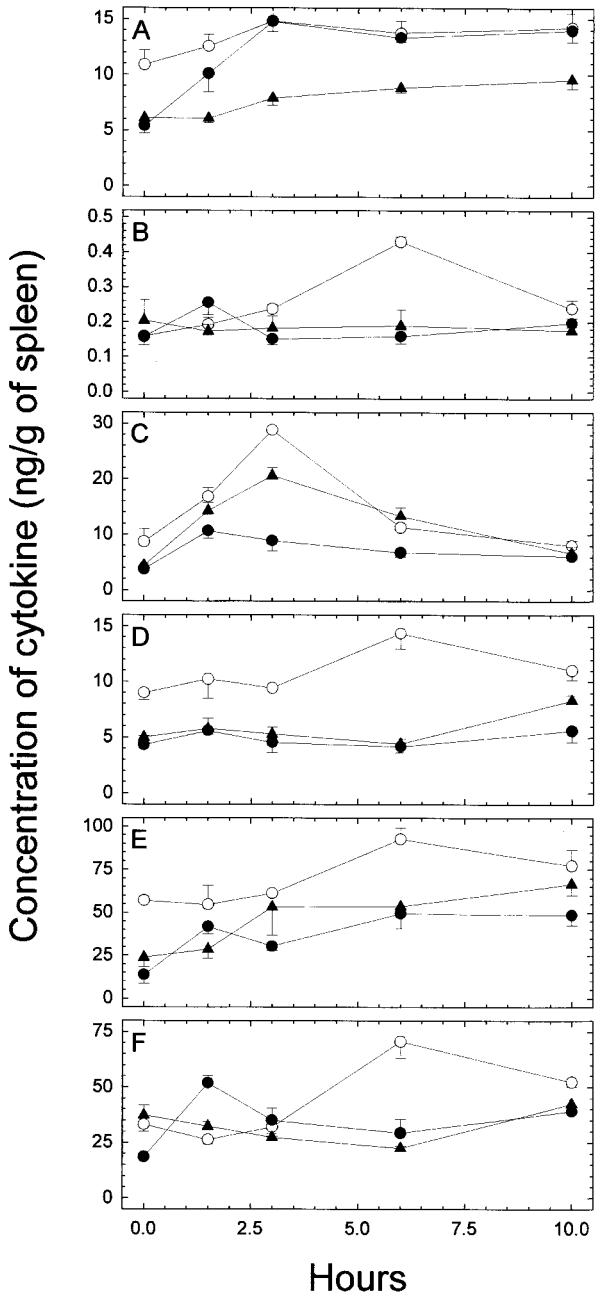

FIG. 5. Concentrations of IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), IFN-γ (E), and TNF-α (F) in spleens of mice receiving an initial intraperitoneal injection of 100 μg of BSA in 0.1 ml of PBS (○), 5 μg of LPS in 0.1 ml of PBS (●), or 50 μg of V_h in 0.1 ml of PBS (▲) followed in 48 h by an intraperitoneal injection of 200 μg of LPS in 0.1 ml of PBS (at which time the determination was initiated).

vent release of free NF-κB, which is known to favor transcription of proinflammatory cytokine mRNA (4, 5, 37, 66, 67).

The results of the present study are in accord with the generally accepted precept that marrow and visceral organs such as the spleen serve as primary sources of both anti- and proinflammatory cytokines. Consequently, cytokines in adjacent compartments such as the PLF and blood may reflect spillover from primary compartments and thus exhibit marked decreases in concentration. This variable as well as potential differences in half-life tend to focus attention on primary sources of cytokine synthesis as indicators of changes mediated by regulatory agents such as V_h. It is important, however, to determine the kinetics of cytokine expression in secondary pools to define the steady state and appreciate possible secondary regulatory effects (e.g., putative up-regulation of IL-10 at distal sites by TNF-α in blood). Another concern in evaluating data from whole animals is interpreting the significance of determinations where injection of one reagent promotes up-regulation whereas concomitant injection of another does not (e.g., amplification of IL-10 by V_h but not by V_h plus LPS).

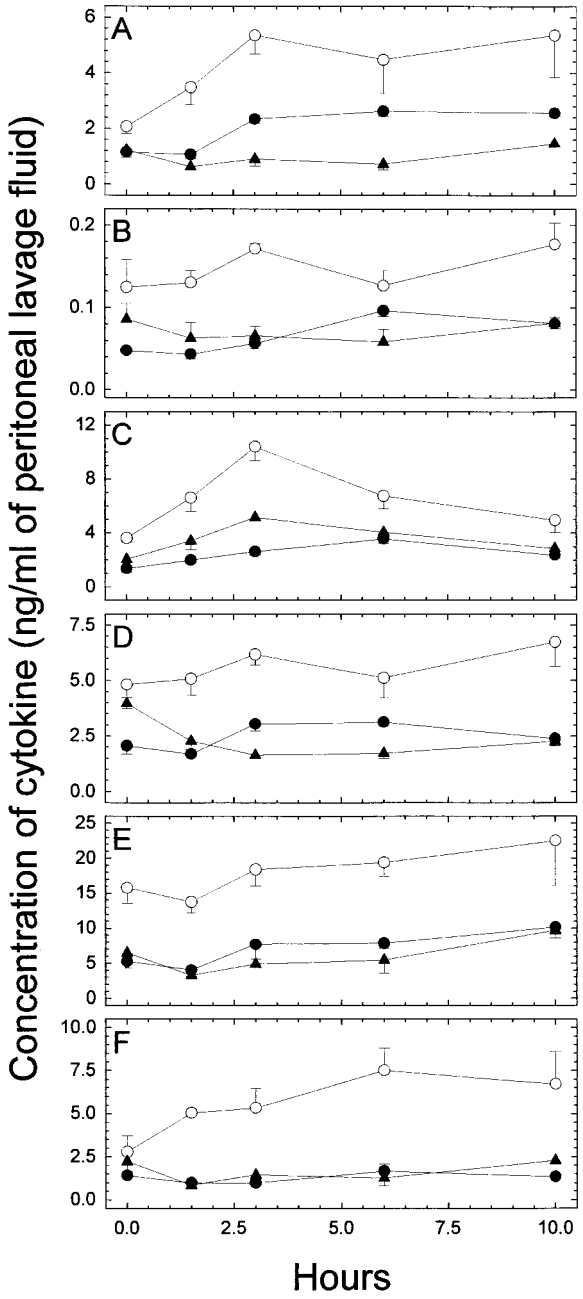

FIG. 6. Concentrations of IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), IFN-γ (E), and TNF-α (F) in the PLF of mice receiving an initial intraperitoneal injection of 100 μg of BSA in 0.1 ml of PBS (○), 5 μg of LPS in 0.1 ml of PBS (●), or 50 μg of V_h in 0.1 ml of PBS (▲) followed in 48 h by an intraperitoneal injection of 200 μg of LPS in 0.1 ml of PBS (at which time the determination was initiated).

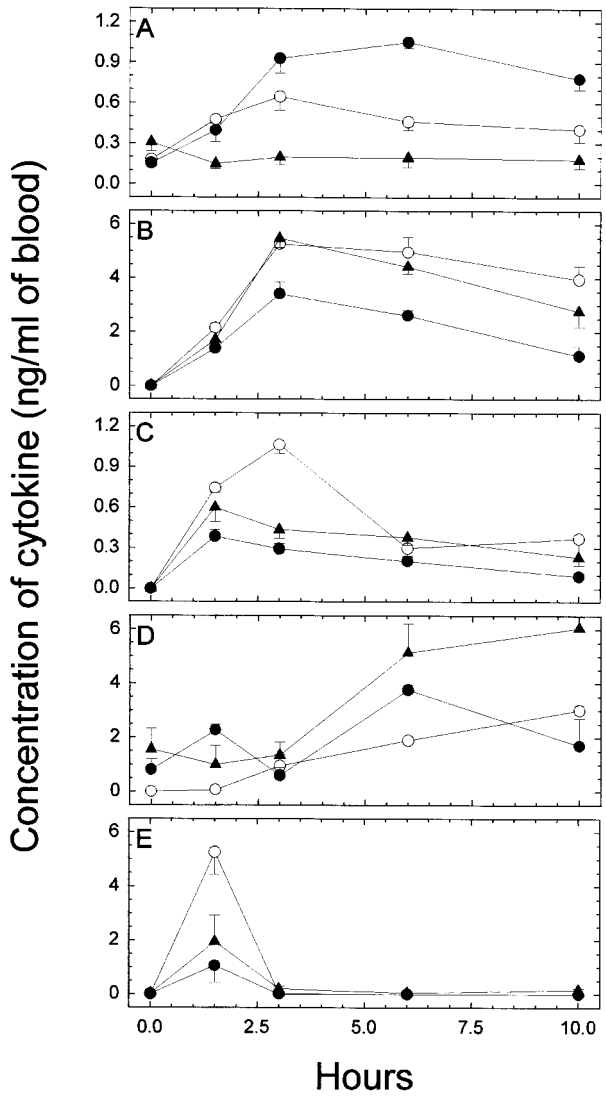

FIG. 7. Concentrations of IL-2 (A), IL-6 (B), IL-10 (C), IFN-γ (D), and TNF-α (E) in blood of mice receiving an initial intraperitoneal injection of 100 μg of BSA in 0.1 ml of PBS (○), 5 μg of LPS in 0.1 ml of PBS (●), or 50 μg of V_h in 0.1 ml of PBS (▲) followed in 48 h by an intraperitoneal injection of 200 μg of LPS in 0.1 ml of PBS (at which time the determination was initiated).

As already noted, IL-10 protects against the lethal effect of LPS by down-regulating those proinflammatory cytokines, primarily IL-1, IFN-γ, and TNF-α, which account for endotoxic death. Two lines of evidence presented in this report indicate that V_h provides protection against the lethal effect of LPS by amplifying IL-10 (and possibly IL-4). First, a single intraperitoneal injection of 50 μg of V_h provided immediate up-regulation of IL-10 (and some IL-4) in the spleen as well as modest synthesis of IL-2 and TNF-α (but not IL-6 or IFN-γ). A different pattern was observed after lethal intraperitoneal injection of LPS (200 μg), which failed to up-regulate IL-10 in the spleen but promoted immediate synthesis of IL-2, IL-6, and TNF-α plus marked but delayed expression of IFN-γ. The significant titer of the latter (~500 ng/g by 6 h) undoubtedly contributed to endotoxic death. Of interest was the observation that the nonlethal combination of V_h (50 μg) and LPS (200 μg) inhibited early expression of IL-2, IL-4, and IL-6 in the spleen and prevented significant net synthesis of IFN-γ and TNF-α. This combination, however, did not significantly up-regulate IL-10, suggesting that the latter may have been consumed. All tested cytokines except TNF-α were rapidly expressed in the PLF of mice receiving LPS alone, whereas concomitant injection of V_h markedly inhibited the initial concentrations of these cytokines as well as TNF-α. This pattern was maintained for IFN-γ and TNF-α in blood. It is of interest that the ability of V_h to so modify the kinetics of cytokine synthesis following treatment with LPS closely resembles that reported for the anti-inflammatory drug chlorpromazine (24, 36, 58), which, at least in part, functions by interaction with dopamine receptors (58).

A second line of evidence indicating that V_h induces IL-10 is its ability to generate a delayed phase of resistance to LPS, which peaked at 48 h. This phenomenon mimicked endotoxin tolerance in that primary induction of sublethal levels of proinflammatory cytokines occurred which probably induced subsequent generation of IL-10. As already noted, TNF-α can up-regulate IL-10, and marked levels of the former were immediately expressed upon initial injection of both LPS and V_h. It is probably significant in this context that patterns of cytokine synthesis observed in mice first receiving V_h and then an otherwise lethal dose of LPS and in those receiving both a first and second round of LPS were remarkably similar, especially in PLF. This resemblance was not due to LPS contamination of V_h, as shown by the absence of detectable LPS in V_h, the ability of V_h (but not LPS) to immediately induce significant IL-10, and the observation that boiling or repeated thawing of V_h destroyed anti-inflammatory activity.

The results presented here are consistent with a primary role of V_h as inducer of IL-10 by two distinct mechanisms. These consist of an immediate but unknown process of up-regulation possibly shared by chlorpromazine and by a second delayed procedure probably analogous to endotoxin tolerance. The possibility also exists that IL-4 also undergoes primary amplification by injected V_h; further work will be required to define the extent of the contributions made by these two anti-inflammatory cytokines. Following our discovery that mice infected with Lcr^+ yersiniae fail to express IFN-γ or TNF-α (41), others reported that these organisms can also down-regulate the proinflammatory cytokines IL-8 (52) and IL-12 (9). Since expression of these cytokines is also inhibited by IL-10 (37), the possibility that V antigen also mediates their suppression exists. This mechanism is not, of course, limited to Lcr^+ yersiniae: a variety of other infectious species amplify IL-10 to down-regulate proinflammatory cytokines and thereby survive in vivo (16, 22, 26, 35, 43, 53).

Anderson et al. (2) noted that the mechanism whereby anti-V antigen protects against plague is unknown. This assessment is incorrect: as noted above, anti-PAV restored the ability of mice to express IFN-γ and TNF-α, thereby permitting containment of the organisms within protective granulomas. The results of this study suggest that neutralization of V antigen by a specific antibody prevents up-regulation of IL-4 and, especially, IL-10, thereby enabling the host to express a normal component of proinflammatory cytokines. An explanation accounting for the molecular basis of IL-10 amplification is, of course, beyond the scope of the present manuscript. However, it is predictable from the results presented above that anti-IL-10, like concomitant injection of IFN-γ and TNF-α (41), will protect mice against experimental plague (but prevent V_h from blocking endotoxic shock).

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