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IL-1 GENE EXPRESSION IN LYMPHOID TISSUES[1]

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We examined the expression of IL-1 mRNA in vivo by in situ hybridization. RNA probes for murine IL-1α and IL-1β were used to detect IL-1 mRNA in frozen sections of spleen, lymph node, and thymus of mice injected with *Salmonella typhi* LPS or SRBC. No IL-1 was detected in lymphoid tissues from uninjected mice. This lack of expression correlated with the absence of IL-1 biologic activity. However, after LPS injection, IL-1α and β mRNA expression was found in macrophages of the red pulp and marginal zone of the spleen. The periarteriolar lymphoid sheath contained cells that only expressed IL-1β mRNA. These cells were not lymphocytes and did not stain with the macrophage marker F4/80. A similar cellular response was found after SRBC injection. Scattered macrophages in lymph nodes and thymus were positive, but only after LPS or SRBC injection. The spleens of mice injected with LPS had megakaryocytes containing IL-1 mRNA.

In this paper, we examine IL-1α and -β expression in murine lymphoid organs before and after stimulation with LPS or SRBC. Most studies of lymphoid activation and cytokine production have been performed in vitro under controlled conditions. The correlation of these results with events that occur in vivo has not been entirely defined. In situ hybridization allows the exploration of tissue microenvironments where different cytokines are generated.

MATERIALS AND METHODS

Mice. Female CBA/J mice of 20 to 24 wk of age were purchased from The Jackson Laboratory (Bar Harbor, ME).

Antibodies and reagents. The anti-murine IL-1α antibody was produced by the hamster-mouse hybridoma ALF 161.1 (1) and prepared by protein A purification. A macrophage/monocyte specific antibody F4/80 (2) was used as a culture supernatant and was the generous gift of Dr. Kenneth Tung. *Salmonella typhosa* LPS was obtained from RIBI Laboratories (Hamilton, MT). RPMI 1640 medium was supplemented with 2 mM L-glutamine, 100 μg penicillin, and 100 μg streptomycin (all from GIBCO Laboratories, Grand Island, NY) and 10% newborn calf serum (Hy-Clone Laboratories, Logan, UT).

Assay for IL-1. IL-1 activity was assayed by the growth of the IL-1-dependent T cell clone DM, a mutant derived from D10.G4.1 (3, 4). DM cells (1×10^4) were added to wells containing freeze-thaw lysates of 2×10^5 peripheral blood leukocytes or spleen cells, in a final volume of 200 μl. Cellular IL-1 activity was quantified by measuring [^{3}H]thymidine uptake during the final 16 h of a 72-h culture. Each determination was performed in triplicate and expressed as the mean ± SE. The background values varied between 1,600 and 2,700 cpm with maximal responses using IL-1β standards between 78,000 and 102,000 cpm.

For in situ hybridization, peripheral blood leukocytes were prepared from whole blood by centrifugation over Ficoll-Paque (Pharmacia, Piscataway, NJ). The cells were then washed and centrifuged at 1000 rpm for 6 min onto poly-L-lysine-coated slides.

Tissue preparation. CBA/J mice received i.v. 10 μg of LPS or 5×10^6 SRBC in 0.1 ml sterile pyrogen-free saline. The secondary immune response was studied by subsequent i.v. immunization with 5×10^6 SRBC 10 days after the primary inoculation. At the indicated times after stimulation, mice died and were perfused with 10 ml of pyrogen-free saline followed by 20 ml of 4% paraformaldehyde (5). Organs were then harvested and postfixed in 4% paraformaldehyde with 15% sucrose at 4°C for 16 h. Tissues were frozen in liquid nitrogen, and 4-μm frozen sections were placed onto subbed, poly-L-lysine-coated slides, dehydrated in absolute alcohol, and desiccated for 16 h at room temperature. Tissue sections were then prehybridized and hybridized, or stained by immunoperoxidase.

In situ hybridization. In situ hybridization was performed on tissue sections and cell monolayers according to the method of Angerer and Angerer (5). The cells were treated with 10 μg/ml proteinase K for 5 min at 37°C, washed with filtered deionized water, acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min at room temperature, rinsed in 2× SSC, and dehydrated in ascending ethanol concentrations. The slides were desiccated at room temperature overnight followed by hybridization at 55°C for 16 h. The hybridization solution contained 50% formamide, 10 mM Tris-HCl (pH 7.5), 300 mM NaCl, 1 mM EDTA, 1× Denhardt's solution, 10% dextran sulfate, 500 μg/ml tRNA, 10 mM DTT,[2] and 5×10^6 cpm/ml ^{35}S-labeled IL-1 single stranded RNA probe. After hybridization, the slides were washed in 2× SSC at room temperature for 40 min. They were then incubated at 37°C with 20 μg/ml RNAse A for 30 min, washed in descending concentrations of SSC containing 1 mM DTT, incubated at 55°C in 0.1× SSC:1 mM DTT for 30 min, and rinsed in 0.1× SSC:1 mM DTT at room temperature. Subsequently the slides were dehydrated in increasing concentrations of ethanol and coated with NTB2 photographic emulsion (Kodak). The emulsion was exposed for 10 days at 4°C, and developed with D19 developer (Kodak). Finally, tissue sections and cell monolayers were stained with hematoxylin and eosin.

Probe preparation. The pMuIL-1α and pMuIL-1β plasmids containing 1.3-kb inserts of IL-1α and IL-1β cDNA were the generous gift of Dr. P. Gray, Genentech, Inc. (6). The IL-1α and IL-1β inserts were excised with *Eco*RI and *Xba*I and ligated into the pGem3z vector (Promega). The pGem3z vector containing either IL-1α or IL-1β cDNA was linearized by *Eco*RI and isolated on low melting point agarose. Single stranded ^{35}S-labeled cRNA probes were synthesized from the SP6 promoter, and were subsequently hydrolyzed to 50 to 300 bp (7). Sense strand IL-1α and IL-1β probes were prepared as above except that they were linearized with *Xba*I and synthesized from the T7 promoter. Probe specificity was tested by Northern blot analysis of RNA from LPS-stimulated P388D$_1$ cells; only the expected 2.0-kb IL-1α and 1.3-kb IL-1β mRNA bands were identified by IL-1α and IL-1β antisense probes, respectively. No bands were detected by the sense strand probes, and there was no cross-hybridization of IL-1α cRNA to IL-1β mRNA or vice versa. The antisense probe for murine LCA was prepared as described for IL-1 probes. The pGEM3 vector containing a 1.8-kb *Eco*RI fragment of p70Z/3 was the generous gift of Dr. M. Thomas (8).

Combination immunoperoxidase staining and in situ hybridization. The avidin-biotin-peroxidase complex method was used for staining (9). Briefly, frozen sections were incubated with 1% goat serum (Hazelton, Lenexa, KS) in PBS for 20 min at room temperature, followed by sequential incubations with the anti-macrophage-specific rat mAb F4/80 or an irrelevant rat antibody and biotinylated,

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[1] This work was supported by grants from the National Institutes of Health.

[2] Abbreviations used in this paper: DTT, dithiothreitol; LCA, leukocyte common Ag; PALS, periarterial lymphoid sheath.

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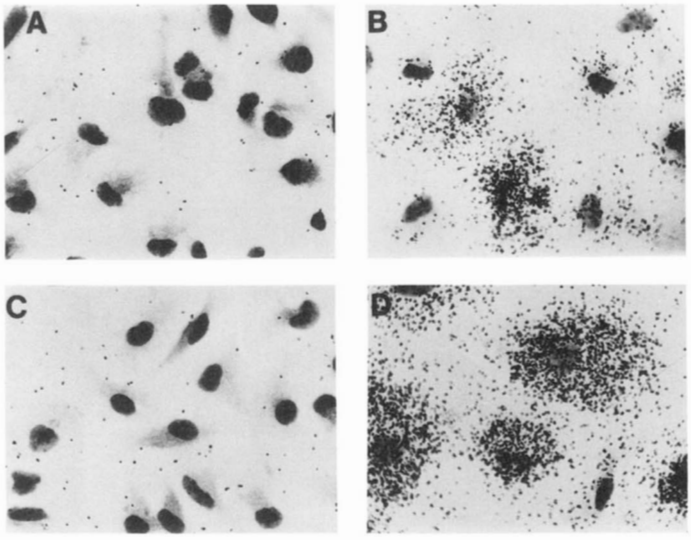

Figure 1. In situ hybridization of cultured peritoneal macrophages with IL-1α (*A* and *B*) and IL-1β (*C* and *D*) antisense probes. Peritoneal macrophages were cultured for 18 h in medium. After additional culture for 4 h in the presence of medium (*A* and *C*) or 10 μg/ml LPS (*B* and *D*), the macrophages were washed, fixed, and processed for in situ hybridization.

mouse adsorbed, rabbit anti-rat Ig (Vector Laboratories, Burlingame, CA), for 30 min at room temperature. Endogenous tissue peroxidases were quenched by a 30-min room temperature incubation with 3% H_2O_2 in saline. Finally, the sections were incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 30 min at room temperature and developed in a diamino-benzidine-H_2O_2 solution for 9 min. Tissue sections were then hybridized in situ as described above and counterstained with methyl green (Rowley Biochemical, Rowley, MA). Important alterations in the standard immunoperoxidase staining protocol included two steps to prevent RNAse activity: the addition of diethylpyrocarbonate to the PBS (0.04% solution of pyrocarbonic acid diethyl ester (Sigma) in PBS was incubated at 37°C for 18 h and then autoclaved before use); and the presence of 5000 U/ml heparin in goat serum. In addition, tissue sections were immersed in 4% paraformaldehyde immediately after proteinase K treatment during the in situ hybridization procedure. Comparison of spleen sections stained by immunohistochemistry alone to sections treated by immunohistochemistry and in situ hybridization showed identical F4/80 staining. Although there was some decrease in the hybridization signal when the combined technique was used, the tissue distribution of mRNA was identical to that observed with in situ hybridization alone. In every experiment the controls included: 1) the use of sense RNA probes, which never gave a positive signal; 2) the examination of slides with peritoneal macrophages cultured in media—these were F4/80-positive and IL-1α- and IL-1β-negative and did not give a positive signal either with the sense or antisense RNA probes; and 3) peritoneal macrophages cultured for 4 h in 10 μg/ml LPS, which were positive for F4/80, IL-1α, and IL-1β (Fig. 1)—these always gave a positive signal with antisense RNA probes.

RESULTS

IL-1 expression in the spleen after LPS. IL-1 was not identified in spleen, lymph nodes, or thymi of normal mice. Controls for hybridization conditions (IL-1α sense strand) and RNA stability (LCA antisense strand) were used in every experiment. As shown in Figure 2, hybridization with IL-1α sense probe showed no positive cells, and established the low level of background hybridization. In contrast, most cells hybridized with the LCA (CD45) antisense probe, indicating uniform preservation of RNA throughout the tissue; mRNA for LCA was present in all nucleated cells of hematopoetic origin.

TABLE I

IL-1α and IL-1β mRNA expression in CBA/J spleen

Data are presented as the relative numbers of cells expressing IL-1 mRNA within a given area of spleen. Positive cells bear 50 to 200 grains. Animals were given 10 μg of LPS i.v. At the specified times, the mice died and were perfused; spleens were harvested and processed for in situ hybridization. Serial sections of spleens for each time point were hybridized with IL-1α (α) and IL-1β (β) antisense probes. Identical results were obtained in four separate experiments.

Time after i.v. LPS	Red Pulp		Marginal Zone		Periarterial Lymphoid Sheath	
	α	β	α	β	α	β
0 h	–	–	–	–	–	–
0.5 h	+++	++++	–	+	–	+
1 h	+++	++++	+	+	–	++
2 h	+	++	+	+	–	+++
3 h	+	++	+	++	–	++++
4 h	+	+	++	+++	–	++++
5 h	+	+	+++	++++	–	++++
6 h	+	+	+++	++++	–	++++
8 h	+	+	++	+++	–	–

–, No positive cells were identified.
++++, The maximum signal seen in that region.

Transient expression of both forms of IL-1 mRNA was seen after i.v. LPS (Table I). IL-1 was localized to three areas of the spleen: the red pulp, marginal zone, and PALS. First, the red pulp, composed primarily of macrophages in close approximation to the circulation, contained cells of macrophage morphology with high levels of IL-1 mRNA within 30 min of LPS injection (Fig. 3, *panels E* and *F*). By 4 h, very few IL-1 mRNA-bearing cells were observed; most of the positive cells were megakaryocytes and neutrophils. Second, the marginal zone, which surrounds the white pulp and is presumably one site of B cell activation by T independent Ag, did not contain macrophages with high levels of IL-1 mRNA until 4 h after LPS (Fig. 4). This mRNA declined by 8 h. Third, the PALS, the site of T cell proliferation in response to T-dependent Ag, contained cells bearing high levels of IL-1β mRNA from 2 to 6 h. Only IL-1β mRNA was identified in the PALS, in contrast to the marginal zone and red pulp where both forms of IL-1 message were seen (Fig. 4).

Resident cells of the PALS include primarily T cells and dendritic cells with occasional macrophages and B cells. We showed that B cells did not make IL-1 in response to LPS (10). In addition, T cells purified from spleens 4 h after i.v. LPS did not express detectable IL-1

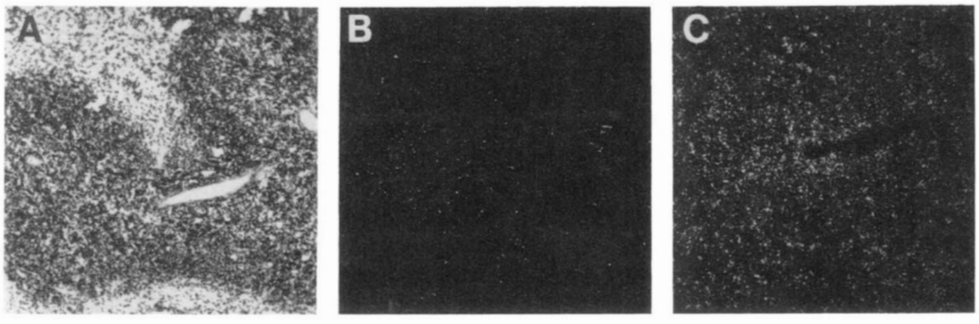

Figure 2. Controls for hybridization conditions of experiments shown in Figures 3 and 4. *A*, Light field image; *B*, IL-1α sense probe shown by darkfield microscopy; *C*, LCA antisense probe by darkfield microscopy. The spleen sections were stained with H&E, and photographed at × 10 magnification.

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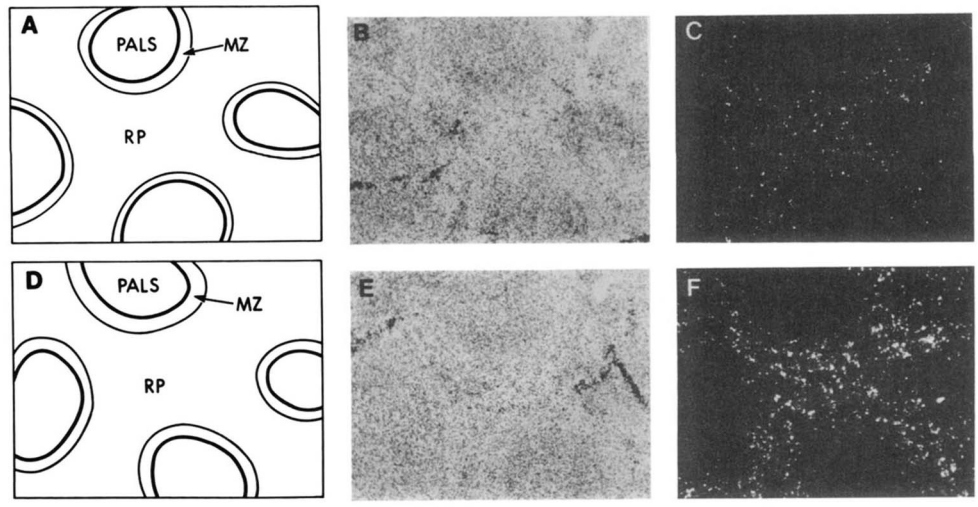

Figure 3. Identification of mRNA in frozen sections of spleen. The distribution of IL-1α (*B* and *C*) and IL-1β (*E* and *F*) mRNA is shown in serial sections at 30 min. *Panels A* and *D* show a schematization of the histology. *Panels B* and *E* show the light field images that correspond to the dark field images shown in *C* and *F*, respectively. *MZ*, marginal zone; *RP*, red pulp.

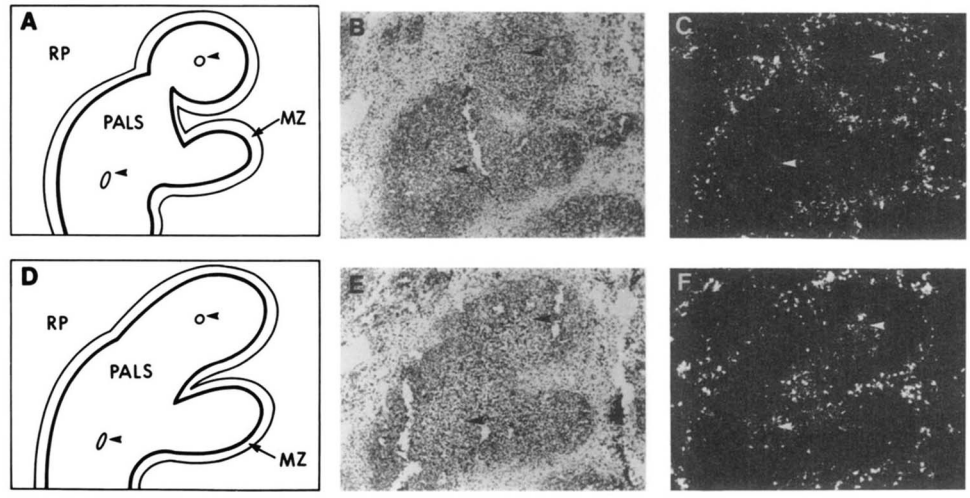

Figure 4. Identification of mRNA in frozen section of spleen. Same as in Figure 3, but 4 h after LPS. Note the IL-1β mRNA-bearing cells in the PALS; this population of cells does not bear IL-1α mRNA (*arrows*). *MZ*, marginal zone; *RP*, red pulp.

mRNA by in situ hybridization. We were unable to stain dendritic cells in tissue sections by immunohistochemistry because the antigenic epitope recognized by NLDC 145 (11) was destroyed by fixation. Sections of spleens harvested 4 h after i.v. LPS and processed by immunohistochemistry followed by in situ hybridization showed that IL-1β-positive cells in the PALS were F4/80 negative (Fig. 5). There was some decrease in IL-1β signal with this double processing, however, obviously positive cells associated with more than 20 grains were identified. The mAb F4/80 stained most cells of the macrophage/monocyte lineage with a few exceptions; dendritic cells were F4/80 negative.

These results were confirmed by direct examination of IL-1 biologic activity. Splenocytes were examined for IL-1 activity at 30 min, 2 h, and 4 h after LPS (Fig. 6). Very little functional IL-1 was present in splenocytes at 30 min, but increased by 2 h and persisted at 4 h. We expected these preparations to show only IL-1α activity (1). This was confirmed by the complete inhibition of T cell proliferation by IL-1α specific mAb (Fig. 6). In other experiments, IL-1 activity was found in the culture supernatants of which only 40% was inhibited by the 161.1 mAb.

Peripheral blood leukocytes were examined for IL-1 protein and mRNA at 30 min, 2 h, and 4 h after LPS. Freeze-thaw lysates of 2×10^6 cells were assayed for IL-

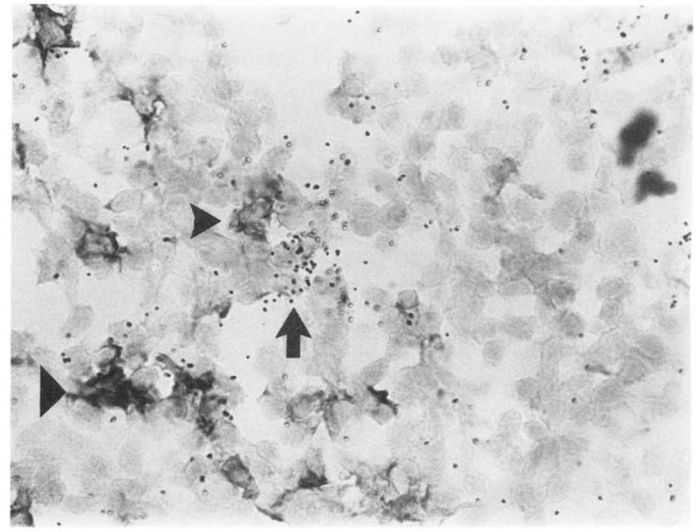

Figure 5. IL-1β mRNA-bearing cells in the PALS are F4/80-negative. Frozen sections of spleen harvested 4 h after injection of 10 μg of LPS i.v. were stained first by immunoperoxidase with F4/80, and subsequently processed by in situ hybridization with IL-1β probe. Shown here is the PALS with F4/80-positive macrophages (*arrowhead*) and IL-1β mRNA-bearing cells (*arrow*). The IL-1β-positive cell shown here is F4/80-negative.

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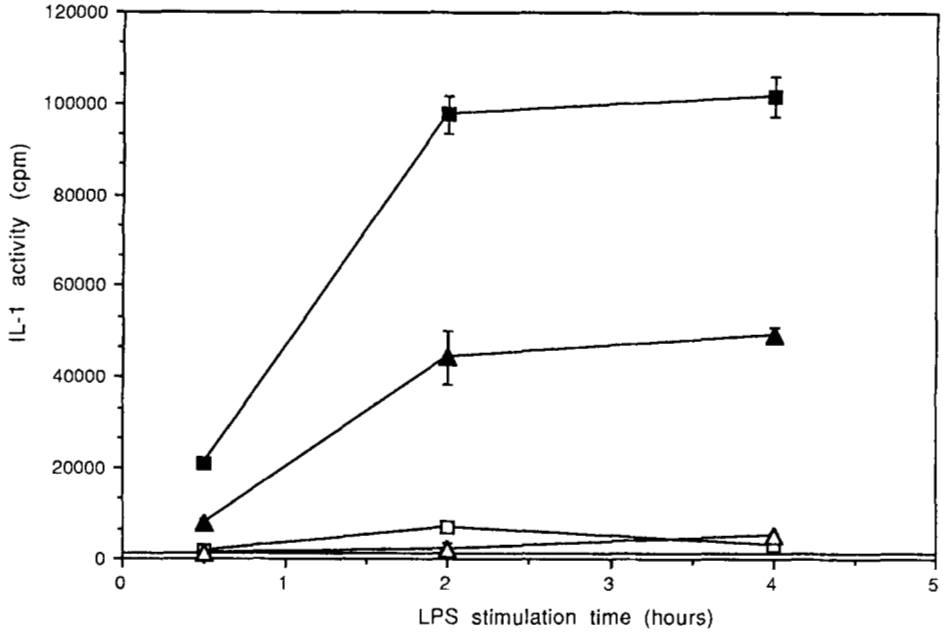

Figure 6. LPS induction of IL-1 in peripheral blood leukocytes (*PBL*) and splenocytes. Freeze-thaw lysates of 2×10^5 PBL (■, □) or splenocytes (▲, △) were assayed for IL-1 induction at 30 min, 2 h, and 4 h after in vivo LPS stimulation. DM cell proliferation assays were performed in the absence (■, ▲) or presence (□, △) of 161.1, a mAb specific for IL-1α. The data points represent the mean ± SE of [^{3}H]thymidine incorporation of triplicate DM cell cultures. Control values were: medium alone, 2036 ± 434 and, medium + 10 μg/ml rIL-1α, 77823 ± 3214.

1 activity (Fig. 6). Low but detectable amounts of functional IL-1 were found by 30 min. By 2 h, there was a dramatic increase in cell-associated IL-1 activity, which persisted at 4 h.

No IL-1 mRNA was found in PBMC of normal mice, however, by 30 min after i.v. LPS approximately 30% had a detectable signal for both forms of IL-1 (Fig. 7). The percent of blood mononuclear cells bearing IL-1 message remained relatively constant at 2 and 4 h after i.v. LPS. Approximately 30% of blood leukocytes had IL-1 mRNA, however the levels were lower (10 to 50 grains/cell) than those observed in splenocytes (20 to 200 grains/cell). In contrast, only 10 to 15% of splenocytes showed IL-1 mRNA, but at much higher levels than in peripheral blood cells.

Our experiments examining LPS-induced IL-1 expression by splenocytes and peripheral blood leukocytes revealed kinetics similar to those previously described for macrophages in vitro (3, 12). Both forms of IL-1 message were detected by 30 min after LPS stimulation, whereas functional IL-1 protein was found by 2 h. These results are consistent with in vitro findings that LPS induced IL-1 expression in cells of the macrophage/monocyte lineage, and not T cells or B cells (10, 13).

IL-1 expression in lymphoid and non-lymphoid organs. We examined tissue sections of lymph node and thymus and also from some non-lymphoid tissues such as kidney, pancreas and liver. There was no difference in the distribution, density, or intensity of positive cells when IL-1α and IL-1β probes were compared (only results with IL-1β probe are shown). No mRNA for either form of IL-1 was detected in organs from normal untreated mice. In contrast, abundant levels of both IL-1α and IL-1β mRNA were seen in macrophages 4 h after i.v. LPS treatment. Popliteal lymph nodes showed high levels of both forms of IL-1 mRNA expressed by macrophages located in the subcapsular and marginal sinuses and around large blood vessels in the cortex (Fig. 8, *A–C*). High levels of IL-1α and IL-1β mRNA were present in macrophages scattered throughout the thymus. More IL-1-positive cells were seen in the thymic medulla than

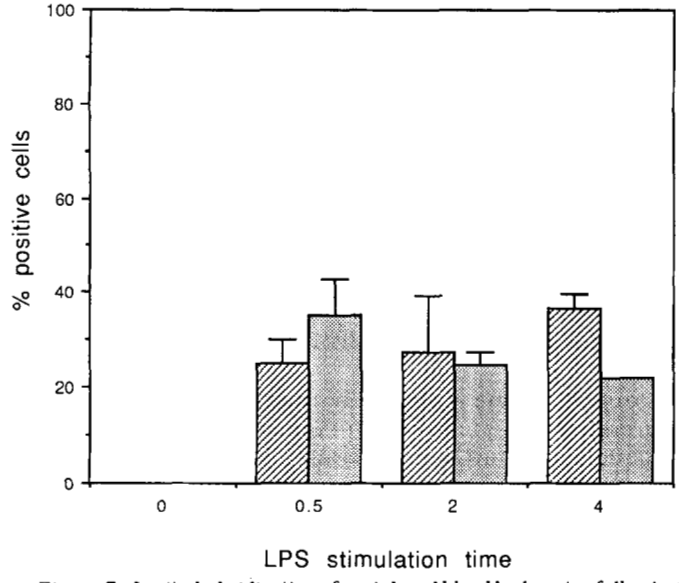

Figure 7. In situ hybridization of peripheral blood leukocytes following in vivo stimulation with LPS. Whole blood was harvested from a normal mouse and from mice at 30 min, 2 h, and 4 h after 10 μg of i.v. LPS. Peripheral blood leukocytes were prepared by centrifugation over Ficoll, washed, and cytocentrifuged onto glass slides. Cell monolayers were processed by in situ hybridization with IL-1α antisense probe (*hatched bars*) and IL-1β antisense probe (*stippled bars*) as described in *Materials and Methods*. These data show the mean ± SE of two separate experiments. The percentage of positive cells represents the percentage of mononuclear cells bearing more than 10 grains out of 200 counted cells. Hybridization with IL-1 sense probe showed no cells with more than five grains at any time point.

cortex, and these IL-1 mRNA-bearing medullary cells were in close approximation to blood vessels (Fig. 8, *D–F*).

Some selected organs were also examined (Fig. 9). LPS induced IL-1α and IL-1β mRNA in macrophages of the renal cortical interstitium. Occasional positive glomerular cells were seen, consistent with previous reports of IL-1 production by mesangial cells (14). No IL-1 mRNA was seen in the renal medulla or in tubular epithelial cells (Fig. 9, *A–C*). Sections of pancreas showed only rare cells bearing high levels of IL-1 mRNA. These positive

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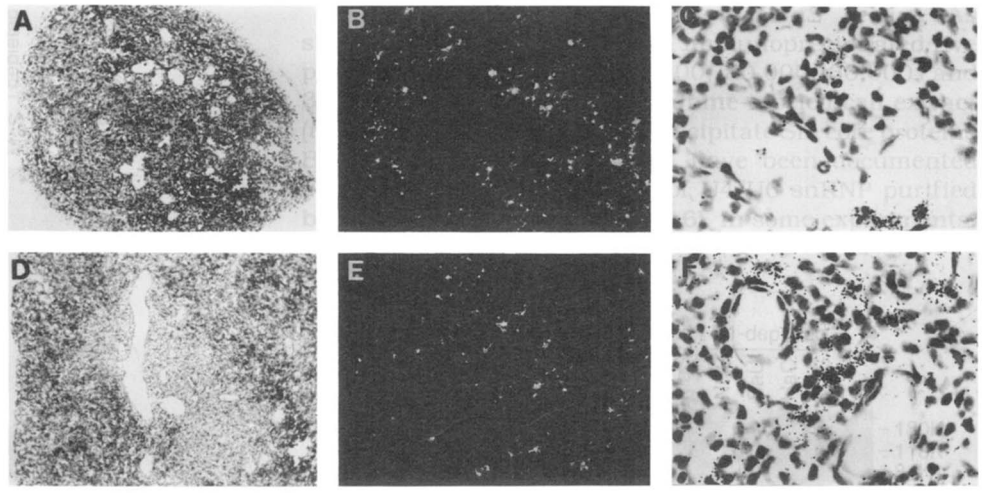

Figure 8. LPS induces IL-1 mRNA in lymph node and thymus. Frozen sections of popliteal lymph node (*A*, *B*, and *C*) and thymus (*D*, *E*, and *F*) harvested 4 h after i.v. LPS were hybridized with IL-1β probe. A single microscopic field was photographed to produce light field (*A* and *D*) and dark field images (*B* and *E*) (× 10 magnification). Collections of white grains in dark field images represent cells bearing IL-1 mRNA; the location of the positive cells may be determined by comparison with the adjacent light field images. Photos taken at higher magnification (× 100) show the close approximation of positive cells to blood vessels (*C* and *F*). Tissue sections were stained with H&E.

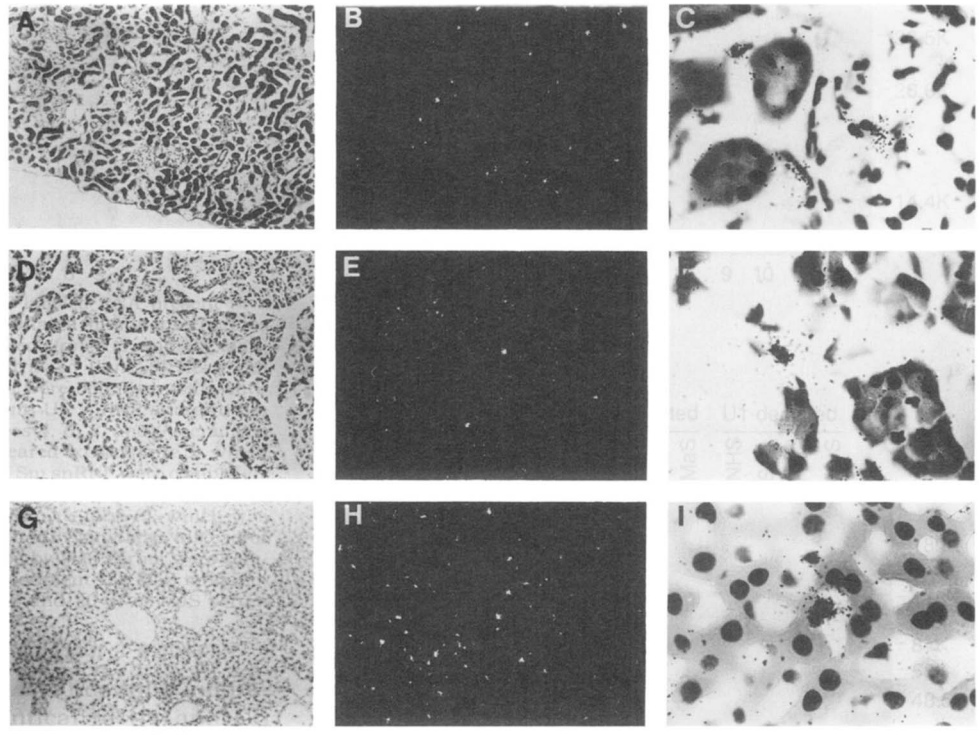

Figure 9. LPS induces IL-1 mRNA in kidney, pancreas, and liver. Frozen sections of kidney (*A*, *B*, and *C*), pancreas (*D*, *E*, and *F*), and liver (*G*, *H*, and *I*) harvested 4 h after i.v. LPS were hybridized with IL-1β probe. A single microscopic field of each organ was photographed to produce light field (*A*, *D*, and *G*) and dark field (*B*, *E*, and *H*) images (× 10 magnification). The location of the positive cells may be determined by comparison with the adjacent light field images. Photographs taken at higher magnification (× 100) show positive cells in a glomerulus and renal tubular interstitium (*C*), a positive cell between pancreatic acini (*F*), and a hepatic Kupffer cell bearing IL-1β mRNA (*I*). Identical results were obtained with the IL-1α probe.

cells were located between the acini of the exocrine pancreas (Fig. 9, *D–F*). (Occasionally an IL-1 mRNA-bearing cell was identified within an islet of Langerhans.) LPS induced abundant levels of mRNA for both forms of IL-1 in hepatic Kupffer cells. IL-1-positive cells were evenly distributed in the hepatic sinusoids; no clustering of positive cells was seen around the portal tracts or central veins (Fig. 9, *G–I*). These findings are consistent with previous reports of LPS induction of Kupffer cell IL-1 in vitro (15).

IL-1 expression in the spleen after SRBC. A similar pattern of IL-1 mRNA expression was found during primary and secondary SRBC responses with transient expression by macrophages in the red pulp and marginal zone. (SRBC were chosen as a representative strong immunogen.) The magnitude of the IL-1 response to i.v. SRBC was less than that to LPS. Many of the macrophages primarily expressed IL-1β mRNA. As with LPS, only IL-1β mRNA was induced in cells of the PALS (Table II). Thymus and lymph nodes were not examined.

DISCUSSION

The major novel result of our experiments is the anatomic compartmentalization of IL-1 expression in the spleen. Macrophages accounted for the response found in the red pulp and marginal zone, which differed in its time of response, whereas a nonmacrophage cell was responsible for that found in the PALS. Cells with only IL-1β mRNA found in PALS are compatible with dendritic cells (16). In vitro studies have reported that isolated splenic dendritic cells did not express IL-1 mRNA or protein (17, 18). In our hands, limited in vitro experiments showed induction of IL-1 mRNA in purified dendritic cells after 4-h culture with LPS. No IL-1α mRNA was expressed, but 60% of dendritic cells expressed high levels of IL-1β mRNA.

The kinetics of IL-1 mRNA expression in the different areas of the spleen could be due to different responses of the cells or to their migration. The identification of IL-1 mRNA initially in red pulp macrophages and later on in marginal zone macrophages suggests that the macro-

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TABLE II

IL-1 mRNA expression in spleen: SRBC response

Data are presented as the relative numbers of cells expressing IL-1 mRNA within a given area of spleen. Positive cells bear 50 to 200 grains. The scale "−" to "++++" is comparable to that in Table I. Mice received 5×10^6 SRBC i.v.; a secondary immune response was generated 10 days later by another i.v. injection of 5×10^6 SRBC. At the specified time, the mice died and were perfused; spleens were harvested and processed for in situ hybridization. Serial sections of spleens for each time point were hybridized with IL-1α (α) and IL-1β (β) antisense probes. Identical results were obtained in five separate experiments.

	Red Pulp		Marginal Zone		Periarterial Lymphoid Sheath	
	α	β	α	β	α	β
Primary SRBC response after i.v. SRBC						
2–3 h	−	+	−	−	−	−
24 h	+	+	−	+	−	−
72 h	+	++	−	++	−	+
5 days	−	+	−	−	−	−
Secondary SRBC response after i.v. SRBC						
0 h	−	−	−	−	−	−
1 h	−	−	−	−	−	−
6 h	+	+++	−	++	−	−
24 h	−	++	−	+	−	++
30 h	−	+	−	−	−	+
48 h	−	−	−	−	−	−

phages in these two splenic compartments may be distinct subsets of cells that respond with different kinetics to LPS stimulation. This is supported by their immunohistochemical staining characteristics; red pulp macrophages are F4/80-positive and ERTR9-negative, whereas marginal zone macrophages are F4/80-negative and ERTR9-positive (Refs. 19 and 20, respectively). Alternatively, macrophages seen in the red pulp may be recently arrived circulating cells stimulated by LPS, before the stimulus has reached the resident tissue macrophages of the marginal zone. It is also possible that red pulp macrophages after LPS stimulation migrate into the marginal zone from the red pulp. This scenario is unlikely, inasmuch as immunohistochemical staining did not show an increase in F4/80-positive cells in the marginal zone after i.v. LPS.

Marginal metallophils are a subset of marginal zone macrophages originally described in the rat (21). They stain by silver impregnation, hence the name, and are MOMA-1-positive, ERTR9-negative, and F4/80-negative (22). Although LPS has been shown to induce migration of marginal metallophils into the PALS (23), it is clear that the IL-1β mRNA-bearing cells in the PALS are not marginal metallophils. First, we did not find either F480-positive or MOMA-1-positive cells in the PALS at 4 h after stimulation. Second, LPS induced marginal metallophil migration at 24 h, but not by 6 h after injection (22).

It has been suggested that marginal zone macrophages may have an important role in the presentation of T-independent Ag to B cells (24–26). We now show that marginal zone macrophages express high levels of IL-1 mRNA in response to the T-independent Ag LPS and also to SRBC. The IL-1 produced by marginal zone macrophage may interact with marginal zone B cells either directly or indirectly via the induction of other cytokines including IL-6, IFN-γ, and IL-2. In particular, the induction of IL-2 by IL-1 may play a key role in marginal zone macrophage-B cell interactions, because IL-2 is an important modulator of B cell growth and differentiation (10, 27).

One issue is whether in vivo LPS induces IL-1 directly or by way of a cytokine. Previous reports indicate that LPS may induce TNF production, which in turn stimulates cells to make IL-1 (28, 29). We have found that i.v. administered anti-TNF-α antibodies had no effect on LPS induction of IL-1 mRNA in spleen or liver, despite complete neutralization of serum TNF (our unpublished observations, with K. Sheehan and R. D. Schreiber). Although it is possible that neutralization of serum TNF was not an accurate reflection of TNF activity in the tissue microenvironment, the results suggest that a pathway exists for LPS induction of IL-1 mRNA in vivo independent of TNF-α. Of course, it is possible that factors other than TNF mediate LPS induction of IL-1 in vivo.

In contrast to a previous report of IL-1 mRNA expression in tissues from normal mice (30), we did not identify either form of IL-1 mRNA in normal tissues. In fact, the only time that we detected IL-1 mRNA in unstimulated mice was during a period of murine hepatitis virus infection of the mouse colony. Although under these circumstances the mice were not overtly sick, and peritoneal macrophage MHC levels remained low, viral antibody titers were positive and low levels of IL-1 mRNA were detected. Clearly environmental exposure contributes to the baseline level of IL-1 mRNA expressed.

Finally, our experiments indicated mRNA for IL-1α and -β in megakaryocytes in agreement with the recent findings of biologically active IL-1 in platelets (31). The megakaryocytes were only positive in mice given LPS, although in our results with platelets (31) it was found in blood from normal individuals. The differences may be attributed to sensitivities between the two assays.

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