Brief Definitive Report

Transition from Interleukin 1β (IL-1β) to IL-1α Production during Maturation of Inflammatory Macrophages In Vivo

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Summary

In situ production of interleukin 1α (IL-1α) and IL-1β was investigated in Peyer's patches (PP) of mice undergoing an acute bacterial infection with *Yersinia enterocolitica* O8. Synthesis of IL-1β, as determined by immunohistochemistry, was found primarily in monocytes migrating into the inflamed PP. In comparison, synthesis of IL-1α was temporarily delayed by at least 24 h and was only found in mature macrophages, which did not produce detectable levels of IL-1β. This indicates a transition from IL-1β to IL-1α production during maturation of monocytes into inflammatory macrophages, and further emphasizes a dichotomy between IL-1α and IL-1β.

IL-1α and -1β are biochemically distinct polypeptides, produced by activated mononuclear phagocytes and other cell types, that act as mediators to increase host defense mechanisms as well as inflammatory reactions. Despite their significant differences, having only ~26% homology at the amino acid sequence level, both forms of IL-1 share most of their biological activities and recognize the same cell surface receptors (1–3).

The existence of two IL-1 proteins that initiate similar host responses raises questions regarding their actual roles and production in vivo. In experimental models of septic shock, nearly all of the circulating IL-1 is accounted for by IL-1β (4), suggesting that IL-1β primarily acts as mediator of acute systemic responses. In comparison, little is known about the induction and regulation of IL-1α and IL-1β in tissues, where IL-1 production is thought to contribute to fibrosis, tissue matrix breakdown, and the influx of inflammatory cells.

The murine model of yersiniosis (5) offers an attractive experimental approach to study the local production of IL-1α and IL-1β. The infection, caused by *Yersinia enterocolitica* O8, primarily affects the Peyer's patches (PP) of the distal ileum, which are readily accessible to cellular and molecular analysis. Here we show, by using immunohistochemistry, that synthesis of IL-1α and IL-1β in inflamed PP is independently regulated in a cell-specific manner. Our results indicate a transition from IL-1β to IL-1α production as infiltrating monocytes mature into inflammatory macrophages in vivo.

Materials and Methods

Bacteria. A plasmid-bearing *Y. enterocolitica* serotype O8 (strain NCTC 10938) was obtained from the National Collection of Type Cultures, Central Public Health Laboratory (London, England). The bacteria were grown overnight at 24–26°C in tryptone yeast extract glucose (Oxoid Ltd., London, England).

Experimental Infection of Mice. Male BALB/c mice (6–8 wk old, ~40 g) were orogastrically infected with 0.1 ml bacterial suspension containing 10^7 cells. For the duration of the experiment animals were given water and food ad libitum.

Recovery of PP. At different times (0–6 d) after infection, groups of five mice were killed by cervical dislocation. The PP were excised from the small intestine and washed in saline to remove the bacteria entrapped in the mucus. Some of the PP were immediately placed into Tissue Tek OCT compound (Diatec, Hallstadt, FRG), snap frozen using isopentane precooled in liquid nitrogen, and stored at −70°C. Single cell suspensions were prepared by gently passing inflamed PP through a net. Cells were then washed, placed onto acid-cleaned microscopic slides, air dried, and fixed for 20 min with 4% (wt/vol) paraformaldehyde.

Immunoperoxidase Staining. Cryostate sections (5 μm) of frozen PP were thawed onto glass slides, fixed with acetone, rehydrated in blocking solution (6), and incubated (12 h, 4°C) with appropriate dilutions of the first-step antisera: rabbit antiserum (1:10,000) to *Y. enterocolitica* O8 (kindly provided by W. Knapp, Erlangen, FRG), rat antibodies (1:1,000) to mouse Mac 1 (Boehringer, Mannheim, FRG), rabbit antiserum (1:10,000) to murine IL-1α, and rabbit antiserum (1:10,000) to murine IL-1β (7). After thoroughly washing with blocking solution, sections were incubated (1 h at room tem-

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perature) with the biotin-conjugated second-stage antibodies: donkey anti-rabbit IgG and mouse anti-rat IgG (Dianova, Hamburg, FRG). Avidin-biotinylated horseradish peroxidase (POX) kits were used according to the supplier's recommendations (DAKO Diagnostics, Hamburg, FRG), and the POX was visualized with 3-amino-9-ethylcarbazole.

Immunofluorescence (IF) Staining. For IF double staining of the mouse macrophage antigen F4/80 and IL-1 proteins, fixed cells on microscopic slides were incubated (12 h, 4°C) first with rabbit antiserum to either IL-1α or IL-1β and then with the rat anti-mouse F4/80 antibody (kindly provided by S. Gordon, University of Oxford). Labeling was done by sequential incubation (1 h at room temperature) with tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-rabbit IgG and with FITC-conjugated mouse anti-rat IgG (Dianova, Hamburg, FRG). For IF double staining of IL-1, proteins in fixed tissue sections slides were incubated (12 h, 4°C) with rabbit antiserum to murine IL-1β followed by TRITC-conjugated goat anti-rabbit IgG (1 h, 37°C). To block free antibody binding sites of the goat antibody, rabbit serum was added for 30 min at room temperature, followed by digoxigenin-conjugated (DIG) rabbit anti-murine IL-1α IgG (diluted 1:100). Finally, FITC-conjugated sheep anti-DIG Fab (Boehringer) was added. Microscopic slides were mounted in 10% glycerol in PBS.

Modification of Rabbit IgG with DIG. Antiserum to murine IL-1α was affinity purified using protein A–Sepharose (Pharmacia, Freiburg, FRG). The purified IgG was diluted to 1 mg/ml and covalently modified with digoxigenin-3-*O*-methylcarbonyl-e-aminocaproic acid-*N*-hydroxy-succinimide ester according to the supplier's instructions (Boehringer). To remove nonreacted DIG, the protein solution was applied to a Sepharose G25 column.

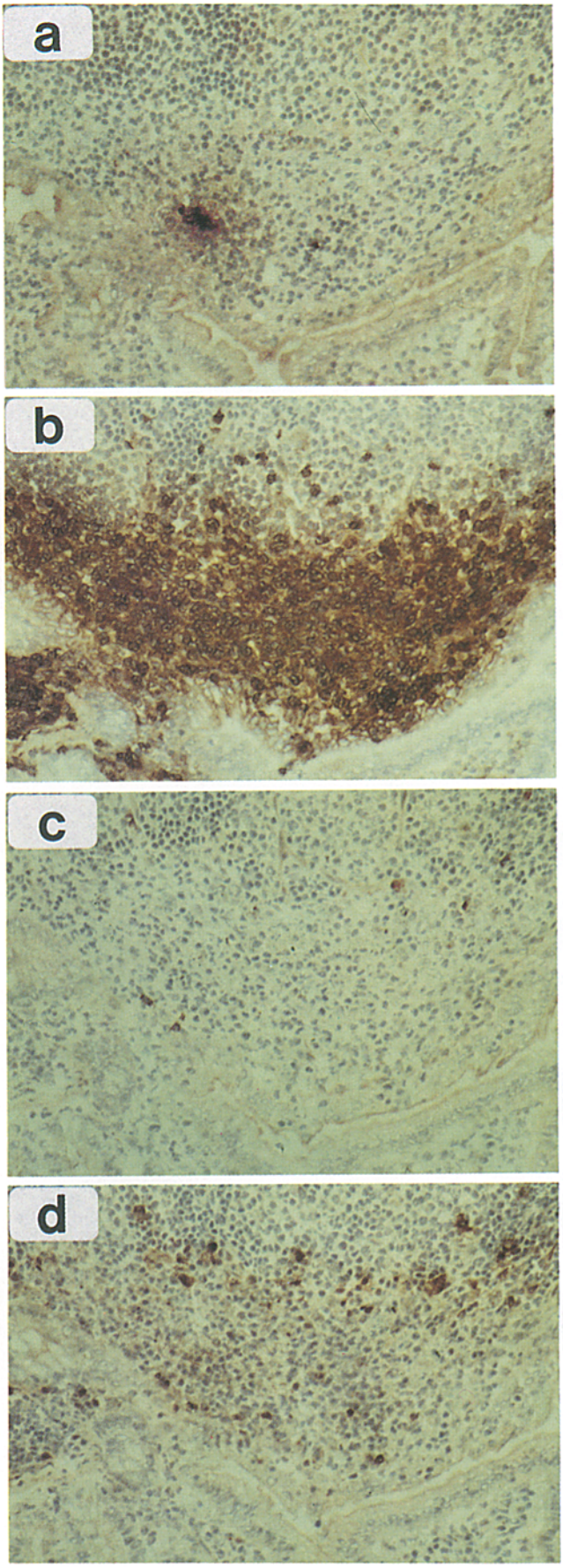
Myeloperoxidase Staining. For myeloperoxidase staining, PP sections were fixed with acetone for 1 min and stained with a benzidine dihydrochloride/Safranin O mixture as described (8).

Microscopic Analysis. Cells and tissue sections were examined using an Axiophot microscope (Zeiss, Oberkochen, FRG) equipped with appropriate filters to gate red and green fluorescence.

Results and Discussion

Immunohistochemical staining of tissue sections using antiserum raised against *Y. enterocolitica* O8 established a bacterial colonization of PP as early as 36 h after oral challenge (Fig. 1 *a*). Bacterial invasion of PP correlated with an infiltration of phagocytic cells (Fig. 1 *b*), as revealed by Mac 1 staining (9). In adjacent sections, determination of IL-1 synthesis using antiserum to either form of IL-1 showed that recruited cells produced IL-1β (Fig. 1 *d*) but not IL-1α (Fig. 1 *c*). Even so, 6 d after oral challenge, staining for both IL-1α (Fig. 2 *a*) and IL-1β (Fig. 2 *b*) was readily detectable in PP. This indicates that in inflamed PP, synthesis of IL-1β is induced before IL-1α. However, the distinct tissue distribution of IL-1α (Fig. 2 *a*) and IL-1β (Fig. 2 *b*) staining indicates that the two IL-1 proteins are produced in a cell-specific manner.

Differential expression of IL-1α and IL-1β mRNA has been observed in some cell types, i.e., human monocytes, T cells, and B cell clones (10). However, immunofluorescence double staining of single cells prepared from inflamed PP revealed that synthesis of IL-1 α (Fig. 3 *a*) as well as IL-1β (Fig. 3 *b*) correlated with the expression of the F4/80 antigen, a surface protein found on murine macrophages (11). In control experiments, synthesis of both IL-1α and IL-1β did not correlate



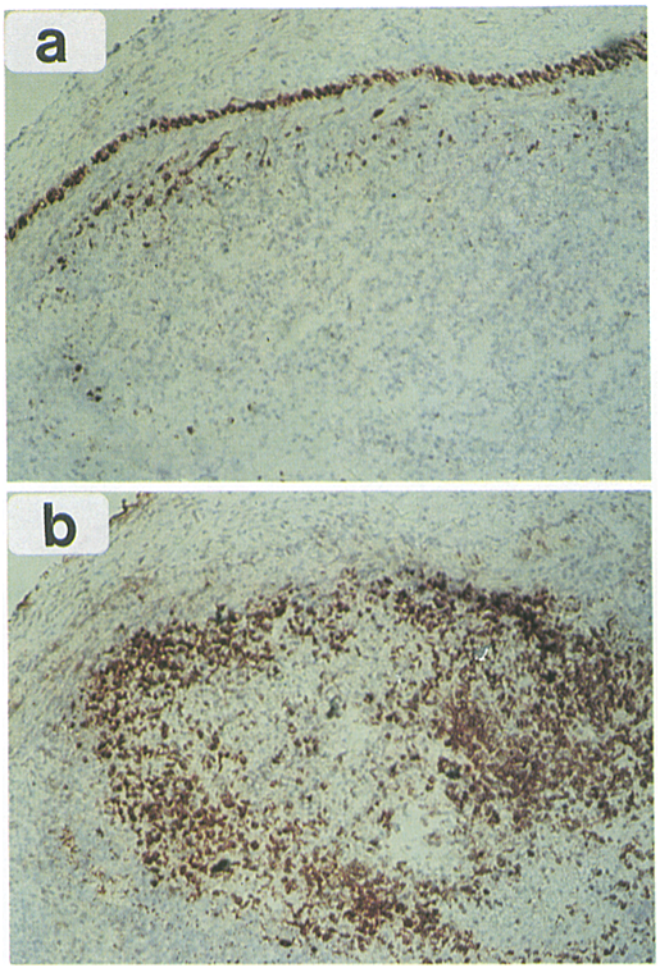


Figure 2. Immunoperoxidase staining of PP tissue removed from mice after 6 d of infection. Photomicrographs (×100) of adjacent sections incubated with (*a*) antiserum to IL-1α and (*b*) antiserum to IL-1β, and counterstained with hematoxylin. IL-1α-producing cells are seen along the lamina muscularis mucosae, whereas cells synthesizing IL-1β are located in the center of the PP.

with staining patterns obtained for surface proteins of T and B cells (data not shown). In addition, no IL-1 synthesis was observed in neutrophils, identified by hematoxylin staining (data not shown). Thus, lack of F4/80 staining on some IL-1β-producing cells (Fig. 3 *b*) is likely to result from a variation in the level of expression of F4/80 antigen (11) on individual macrophages.

Previous studies have suggested that a repression of IL-1β transcription may be acquired by monocytes as they mature into macrophages in vitro (12). To investigate whether the cell-specific production of IL-1α and IL-1β in PP may be related to the state of maturation of mononuclear phagocytes, a PP section was stained for myeloperoxidase, an enzyme found in newly formed monocytes, but not in mature macrophages (8). Positively stained cells were only detectable in the centre of the section (Fig. 3 *c*), a tissue area corresponding to IL-1β, but not IL-1α immunoreactivity (see Fig. 3 *d*). This indicates that in inflamed PP, synthesis of IL-1β is induced in monocytes, whereas cells producing IL-1α are compatible with mature macrophages.

The differences in the kinetics of IL-1α and IL-1β synthesis (Figs. 1, *c* and *d*, and 2, *a* and *b*) in PP can be explained differently. First, recruited monocytes are stimulated to produce IL-1β before the activating stimulus has reached the resident tissue macrophages that produce IL-1α. Second, there is a transition from IL-1β to IL-1α synthesis as recruited monocytes differentiate into inflammatory macrophages. If this, however, is the case, one would expect an intermediate cell type coproducing both forms of IL-1. To address this issue, synthesis of IL-1α and IL-1β in PP was investigated by immunofluorescence double staining. Cells producing either IL-1α or IL-1β are seen by their green or red color, respectively (Fig. 3 *d*). In addition, a few cells produced a yellow image, indicating a coexpression of both forms of IL-1. Detection of IL-1 synthesis was specific, as both IL-1α and IL-1β staining was completely competed by preincubation of the antisera with murine rIL-1α or murine rIL-1β, respectively (data not shown).

Together, these studies have shown for the first time that at a local site of acute inflammation, production of IL-1α and IL-1β is independently regulated in a cell-specific manner. The results are compatible with a transition from IL-1β to IL-1α production as recruited monocytes mature into inflammatory macrophages. Although our studies cannot establish the underlying molecular mechanisms, differences in IL-1α and IL-1β synthesis could result from changes in transcription (13) and mRNA stability induced during cell development. In addition, as suggested by previous in vitro studies (14), differential expression of IL-1α and IL-1β may be regulated, at least partly, by environmental factors, e.g., IFN-γ, GM-CSF, and 1,25-$(OH)_2$-vitamin D_3, which are involved in monocyte differentiation.

Our results may have important biological implications. It has been noted that IL-1β mediates immunomodulation in vivo, whereas IL-1α acts as a negative regulator of the IL-1β activity, by direct competition for receptor occupancy (15). These differential activities of the two IL-1 proteins may be regulated through a sequential production of IL-1β and IL-1α as monocytes mature into inflammatory macrophages. In addition, because IL-1α and IL-1β share most of their biologic activities, a delayed production of IL-1α could serve to compensate the tissue clearance of IL-1β produced at the first place.

Figure 1. Immunoperoxidase staining of PP tissue removed from mice 36 h after oral challenge with *Y. enterocolitica* O8, Photomicrographs (×200) of adjacent sections incubated with (*a*) antiserum to *Y. enterocolitica* O8. (*b*) antibodies to Mac 1, (*c*) antiserum to IL-1α, and (*d*) antiserum to IL-1β. Positive staining is shown by the brown color. A bacterial colony and recruited phagocytic cells are seen under the virtually intact epithelium. IL-1β synthesis is seen in the tissue area corresponding to recruited phagocytic cells. IL-1α synthesis, however, is not detectable. Sections were counterstained with hematoxylin.

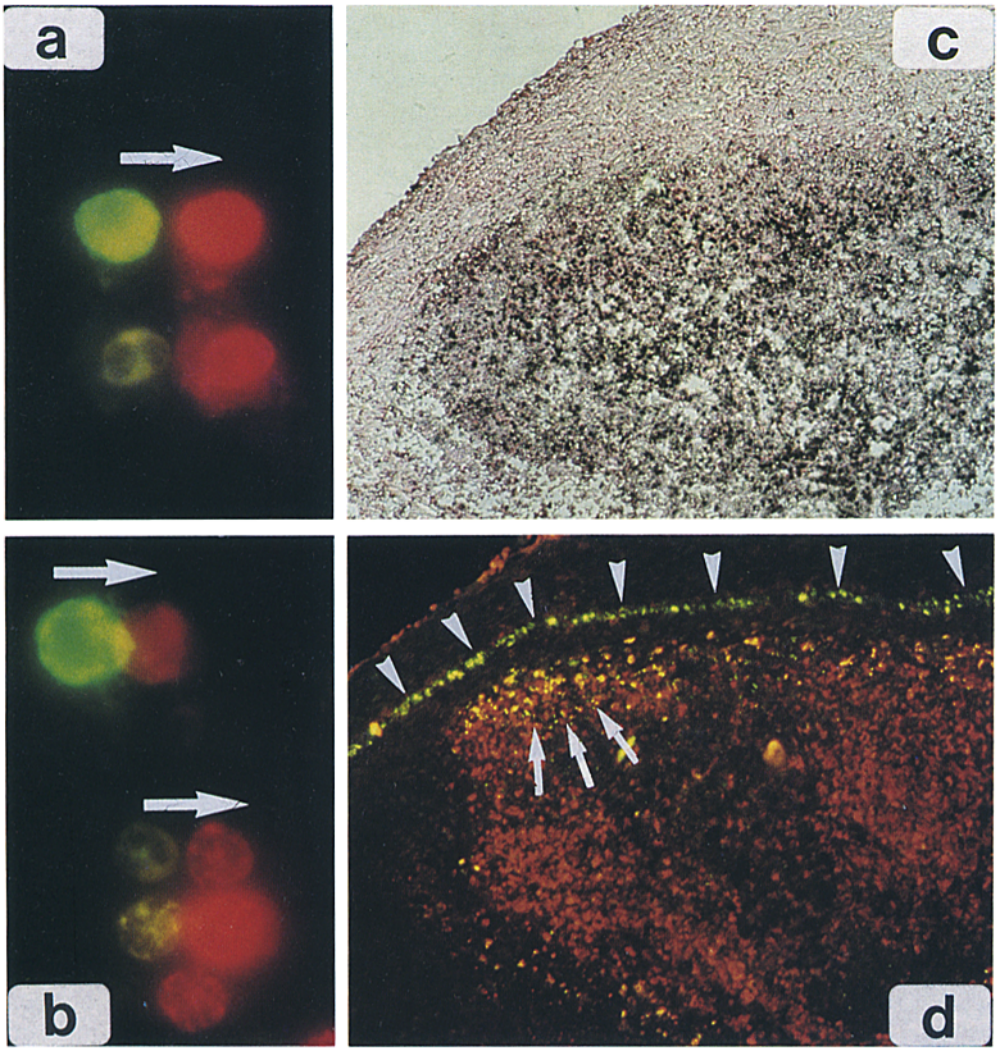


Figure 3. Characterization of IL-1α- and IL-1β-producing cells in PP of mice after 6 d of infection. Immunofluorescence double staining of acetone-fixed cell suspensions of PP using antiserum to either IL-1α (*a*) or IL-1β (*b*) in combination with the F4/80 antibody. Photomicrographs (×1,000) show that cells synthesizing IL-1α or IL-1β (red) express the mouse macrophage antigen F4/80 (green). To visualize both colors on the same cell, slides were gently moved (direction as marked by the arrows) before changing the fluorescence filters. (*c*) Photomicrograph (×100) of an adjacent section as in Fig. 2, but stained for myeloperoxidase, and counterstained with safranin. Cells containing the enzyme activity stain dark blue in the center of the PP. (*d*) Immunofluorescence double staining of an adjacent section as in *c* using antiserum to IL-1α and IL-1β. Cells producing either IL-1α or IL-1β stained green (arrow overheads) or red, respectively. Cells cosynthesizing both forms of IL-1 stained yellow (*arrows*), which results from the mixture of fluorescein and rhodamine.

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