Mice Deficient in IL-1β Manifest Impaired Contact Hypersensitivity to Trinitrochlorobenzene

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Summary

Mice rendered deficient in IL-1β by gene targeting in embryonic stem cells develop and grow normally in a protected laboratory environment. Endotoxin-stimulated peritoneal macrophages from IL-1β–deficient mice showed normal synthesis and cellular release of IL-1α after treatment with 5 mM ATP demonstrating that IL-1β is not necessary for expression and release of the IL-1α isoform. Mice deficient in IL-1β showed unaltered sensitivity to endotoxic shock, with or without pretreatment with D-galactosamine. In contrast, IL-1β–deficient mice showed defective contact hypersensitivity responses to topically applied trinitrochlorobenzene (TNCB). This defect could be overcome either by application of very high doses of sensitizing antigen, or by local intradermal injection of recombinant IL-1β immediately before antigen application. These data demonstrate an essential role for IL-1β in contact hypersensitivity and suggest that IL-1β acts early during the sensitization phase of the response. They suggest an important role for IL-1β in initiation of the host response at the epidermal barrier.

The potent proinflammatory cytokine interleukin-1 (IL-1) is synthesized in two isoforms (designated IL-1α and IL-1β) encoded by distinct, chromosomally linked genes (1–3). Both forms of IL-1 are synthesized as 31-kD intracellular precursors that are cleaved to the 17-kD mature cytokines found in culture supernatants and extracellular fluids (4). The maturation of the IL-1α precursor is thought to be dependent on the calcium-activated proteinase calpain (5), whereas the maturation of the IL-1β precursor is dependent on the cysteine proteinase IL-1β–converting enzyme (ICE)[1] (6). Two cell surface receptors for IL-1 have been defined (7–9), designated IL-1RI and IL-1RII. Mature IL-1α and IL-1β bind similarly to these two defined receptors (10) and, consequently, demonstrate the same activities in a broad range of in vitro and in vivo biological systems.

The ability of the IL-1 proteins to modulate the normal and pathological physiology of essentially all tissues is a consequence of the broad expression of the defined IL-1 receptors. Based on studies of the action of the cytokines in vitro and following their administration to experimental animals in vivo, IL-1 is thought to potentiate inflammatory responses and to promote tissue repair (4). These studies, however, define only the potential actions of IL-1 in normal and pathological responses. Studies defining the physiological responses in which IL-1 acts in an obligatory fashion remain incomplete. Definition of the essential functions of IL-1 in vivo is complicated by the fact that many of the actions of IL-1 defined in vitro are shared with other cytokines, particularly with IL-6, tumor necrosis factor (TNF) and lymphotoxin (LT) (4, 11, 12). Furthermore, in many tissues these cytokines regulate each other's expression, forming a potentially complex and interactive cytokine network (13).

There has been considerable progress in defining the essential functions of IL-1 in vivo by using either neutralizing monoclonal antibodies (against IL-1 or against the IL-1 receptors) (14–16), soluble IL-1 receptors (17), or the naturally occurring IL-1 receptor antagonist (IL-1ra) (18) to block IL-1 action in experimental animal models. Using these approaches, IL-1 has been implicated as a significant mediator in the generation of fever (19) and acute inflammatory responses (20). IL-1 also contributes fundamentally in the normal host response against infection by *Listeria*

[1] *Abbreviations used in this paper:* ES, embryonic stem; ICE, IL-1β–converting enzyme; IL-1ra, IL-1 receptor antagonist; LT, lymphotoxin; PEC, peritoneal exudate cells; TNBS, trinitrobenzene sulfonate; TNCB, trinitrochlorobenzene.

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monocytogenes (16). This action of IL-1 is at least in part dependent on actions of this cytokine in the recruitment of neutrophils and in the activation of macrophages (21).

Few studies of this nature, however, have demonstrated unique roles for either of the IL-1 agonist isoforms. Studies by Geiger et al. (22) and Van den Berg et al. (23) have suggested a selective role for IL-1β in the initiation and progression of collagen-induced arthritis in mice. A fundamental action of IL-1 has also been suggested in a transgenic model of peripheral arthritis due to aberrant expression of TNFα (24).

An additional selective role for IL-1β has been proposed in the development of contact hypersensitivity responses in the skin (25, 26). Contact sensitizers increase the levels of mRNA for several cytokines in the skin, including IL-1α, IL-1β, and TNFα. IL-1β mRNA is increased first, being detected within 15 min after application of the sensitizing agent. Selective depletion of individual epidermal cell populations suggests that Langerhans cells are the primary source of IL-1β, and that keratinocytes are the primary source of IL-1α (27). The regulatory specificity of IL-1β in contact hypersensitivity responses was suggested by studies in which intradermal injection of monoclonal anti-murine IL-1β antibodies prevented primary sensitization (26). In contrast, anti–IL-1α antibodies did not interfere with sensitization.

New insights into the in vivo action of IL-1β have come from studies using mice rendered genetically deficient in this cytokine by gene targeting in embryonic stem cells (28). These studies have demonstrated that IL-1β is an essential mediator of the systemic response following subcutaneous injection of turpentine. Mice deficient in IL-1β failed to demonstrate the typical anorexic and febrile responses that follow this maneuver. They also showed an impaired turpentine-induced acute phase response, with failure of induction of plasma IL-6. Plasma IL-6 levels were, however, indistinguishable in normal and IL-1β–deficient mice after challenge with lipopolysaccharide (LPS), indicating that there was no absolute defect in IL-6 production. This study demonstrated a selective role for IL-1β in activating the systemic response to subcutaneous injection of turpentine, and established unequivocally that IL-1β and IL-1α can act independently in vivo.

We report here the independent production of a mouse strain deficient in IL-1β, and experiments demonstrating an essential action of IL-1β in contact hypersensitivity responses to trinitrochlorobenzene (TNCB). This action of IL-1β appears to be expressed at the early times of local sensitization, as normal contact hypersensitivity responses can be restored by intradermal injection of recombinant murine IL-1β locally immediately before the application of the sensitizing antigen. These data establish a critical role for IL-1β in the initiation of the systemic immune response to this agent after sensitization at epidermal surfaces.

Materials and Methods

IL-1β Targeting Vector. A 4.6-kb EcoRI to EcoRV fragment containing exons 1 through 5 of the IL-1β gene was subcloned from cosmid β7.1 (2) into EcoRI- and SmaI-digested pUC119. A SalI site (*underlined*) and a termination codon (*italics*) were introduced into exon 4 using the method of Kunkel (29) and the mutagenesis oligonucleotide 5′-GGC TGC TTC CAA ACC GTC GAC CTG GGC *TGA* CCT GAT-3′. The altered 4.6-kb IL-1β fragment was removed from this vector by digestion with BamHI and was introduced into the BamHI site of pBluescript KS+ (Stratagene, La Jolla, CA) containing the HSV-thymidine kinase gene under the control of a polyoma promoter and enhancer (30). Finally, a 1.6-kb XhoI fragment encoding neomycin resistance (*neo*^{r}) under the control of the *pgk* promoter (31) was inserted into the SalI site in the opposite transcriptional orientation compared to the IL-1β gene. The final targeting vector was 10.6 kb and contained 3.3-kb of sequence homologous to the IL-1β locus in its 5′ arm and 1.3 kb in its 3′ arm. Plasmid DNA for electroporation was linearized by digestion with NotI.

Generation of IL-1β–deficient Mice. D3 embryonic stem cells (provided by T. Doetschman, University of Cincinnati, OH) were electroporated with the linearized IL-1β targeting vector as previously described (32). Homologous recombinants were selected using 250 μg/ml G418 (GIBCO BRL, Gaithersburg, MD) and 2 μM ganciclovir (Cytovene®; Syntex Laboratories, Palo Alto, CA) and screened by Southern blotting.

Southern Blotting. DNA was isolated from the livers of wild-type, heterozygous, and IL-1β–deficient mice or from expanded colonies of transfected D3 cells as previously described (2, 33) and was digested overnight with BamHI. Digested DNA samples were fractionated on a 1% agarose gel, blotted to a nylon filter and hybridized (34) with a ^{32}P-labeled probe from intron 5. The probe was a 352-bp fragment amplified from the IL-1β locus using the polymerase chain reaction (PCR), using the following oligonucleotide primers: CTC TCT TCC ATA ATA ACA AG and GCA GGA GGC AGG TAG AAC TC. It was labeled with [^{32}P]dCTP using the random priming method (Amersham Corp., Arlington Heights, IL).

Northern Blotting. Peritoneal exudate cells (PEC) were elicited with thioglycollate broth and plated at 1.5×10^6 cells/ml as previously described (35). Following adherence overnight, the PEC were incubated either in the presence or absence of 1 μg/ml *E. coli* LPS for 4 h, and RNA was isolated using a modification of the guanidinium isothiocyanate/CsCl method (36) as described (35). Hybridization was with ^{32}P-labeled near full-length fragments of the IL-1α (37) and IL-1β (38) cDNAs labeled using the random priming method.

Metabolic Labeling and Immunoprecipitation. PEC harvesting, culture, pulse-chase labeling using ^{35}S-met, and immunoprecipitation were performed as previously described (15) with hamster anti–mouse IL-1α and anti–mouse IL-1β monoclonal antibodies 161.1 (14) and B122 (15).

Measurement of IL-1 by ELISA. IL-1α and IL-1β were measured using commercial ELISA kits (Genzyme, Cambridge, MA) according to the manufacturer's instructions. PEC were incubated and culture supernatants and cell lysates were prepared as described for metabolic labeling and immunoprecipitation. Cell lysates were centrifuged for 10 min at 4°C in an Eppendorf model 5414 microcentrifuge before assay. The limit of sensitivity of the IL-1α ELISA was 15 pg/ml, and of the IL-1β ELISA 10 pg/ml.

Flow Cytometry. Single cell suspensions from spleen, thymus, and lymph nodes were prepared from a 7-wk-old IL-1β–deficient mouse and its wild-type littermate. Dead cells and erythrocytes were removed by centrifugation over Ficoll-Hypaque (Sigma Chem. Co., St. Louis, MO) and washed in flow cytometry buffer (HBSS containing 1 mM Hepes, pH 7.35 [GIBCO BRL], and 0.2%

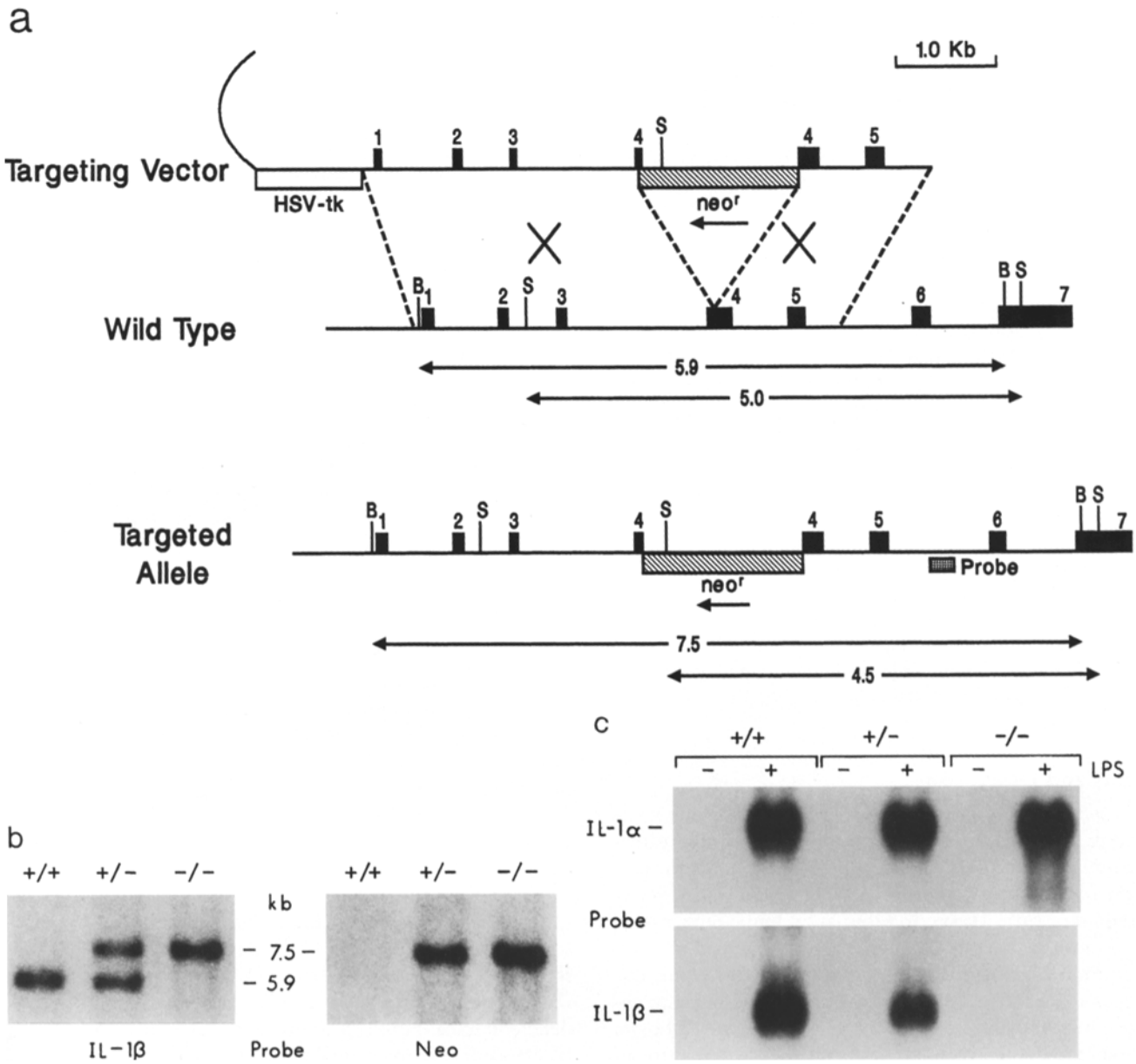

Figure 1. (*a*) Targeted disruption of the gene encoding murine IL-1β. Structure of the targeting vector, the gene encoding the wild-type mouse IL-1β locus and the predicted targeted allele. Black boxes indicate exons of the IL-1β gene. The gene was inactivated by insertion of a neo^r cassette into exon 4. This introduces an in-frame stop codon. Homologous recombination of this construct with the IL-1β locus alters the length of SstI and BamHI restriction fragments detected with the 3′ external probe (*stippled box*). *B*, BamHI; *S*, SstI. The lengths of the indicated restriction fragments are in kilobases. (*b*) Southern blot analysis of the IL-1β locus in IL-1β $^{+/+}$, IL-1β $^{+/-}$, and IL-1β$^{-/-}$ mice. DNA was isolated from liver and digested overnight with BamHI. Hybridization probes were the 3′ external IL-1β probe and a fragment of the neo^r gene labeled with [^{32}P]dCTP, as indicated. (*c*) Northern blot of RNA isolated from LPS-stimulated PEC from IL-1β$^{+/+}$, IL-1β$^{+/-}$, and IL-1β$^{-/-}$ mice. Thioglycollate-elicited PEC were allowed to adhere overnight and were then stimulated with LPS for 4 h. Hybridization probes were near full-length ^{32}P-labeled cDNA fragments of IL-1α and IL-1β as indicated.

sodium azide). 1 × 10^6 cells were stained for 30 min at 4°C with the following antibodies: phycoerythrin-conjugated anti-mouse L3T4 (Becton Dickinson, Bedford, MA), FITC-conjugated anti-mouse Lyt-2 (Becton Dickinson), phycoerythrin-conjugated anti-mouse CD45R (PharMingen, San Diego, CA), and FITC-conjugated anti-mouse CD3-epsilon (PharMingen). The cells were then washed three times with flow cytometry buffer and fixed with 2% paraformaldehyde in PBS. Cells were analyzed on a Becton Dickinson FACScan® using the Lysis I program.

Endotoxic Shock. Endotoxic shock was induced by two different protocols. In the first, age-matched mice were injected i.p. with 20 mg D-galactosamine and 10 μg *E. coli* LPS (0111:B4) in 0.5 ml sterile PBS. Lethal responses were always observed within the first 12 h following injection. Mice that survived were observed for 1 wk and appeared to recover completely. In the second protocol, 8–12-week-old mice received 1.5 mg LPS (0127:B8) in 0.5 ml sterile PBS by i.p. injection.

Contact Hypersensitivity. Reactivity to TNCB was determined

using a modification of the method of Ferguson et al. as previously described (39). Briefly, 8–12-week-old IL-1β deficient mice and their wild-type littermates were prepared by shaving their abdomens and then varying doses of TNCB (Eastern Chemical, Smithtown, NY) were applied in 25 μl olive oil/acetone (1:3). 5 d later, the mice were challenged by injection of 33 μl of 10 mM TNBS (Sigma) into the right footpad. The same volume of PBS was injected into the left footpad as a negative control. 24 h later, footpad swelling was measured by a blinded observer and expressed as the thickness of the right footpad minus the thickness of the left footpad. Where indicated, recombinant murine IL-1β was injected intradermally at the sensitization site immediately before the application of TNCB. Reactivity to oxazolone was determined as previously described (28).

Lymphocyte Proliferative Responses. Lymphocytes were isolated from the axillary and inguinal lymph nodes of TNCB-sensitized mice. Cells from four mice treated in a similar fashion were pooled and 5×10^5 cells were cultured in triplicate in 96-well flat-bottom microtiter plates in 100 μl RPMI 1640 (GIBCO BRL) with 10% heated fetal calf serum, glutamine, 2-mercaptoethanol, penicillin, and streptomycin. Each well also contained from $0–4 \times 10^5$ 2,4,6-trinitrophenyl (TNP)-coupled, mitomycin-C–treated spleen cells from wild-type sv129 mice (Jackson Laboratories, Bar Harbor, ME). After 78 h of culture, 1 mCi (37 kBq) of [^{3}H]thymidine (DuPont NEN, Boston, MA) was added to each well, and 18 h later, cells were harvested using a PhD cell harvester (Cambridge Technology, Inc., Watertown, MA), and incorporation of radioactivity determined by liquid scintillation counting as previously described (39).

Results and Discussion

Generation of IL-1β–deficient Mice. The IL-1β gene is encoded in seven exons (40). To create a mouse strain carrying a null allele at this locus, we generated a targeting vector in which exon 4 was interrupted by insertion of the PGK-*neo* sequence (31). This introduced an in frame termination codon upstream of the ICE cleavage site encoded in exon 5, precluding the translation of the receptor-binding domain of the protein. The vector contained 3.4 kb of sequence homologous to the IL-1β gene upstream of the *neo* cassette, and 1.1 kb of homologous sequence downstream of *neo*, with HSV-*tk* included upstream of the 3.4-kb arm for negative selection (30) (Fig. 1 *a*). After homologous recombination in embryonic stem (ES) cells, the wild-type 5.9-kb IL-1β BamHI fragment was replaced by a 7.5-kb fragment in the targeted allele (Fig. 1 *b*).

This construct was introduced into D3 ES cells by electroporation and homologous recombinants were selected using ganciclovir and G418. Homologous recombinants were detected at a frequency of ~1 in 100 double drug-resistant colonies. One targeted D3 line passed the targeted allele through the germ line. Matings of heterozygous IL-1β–deficient mice confirmed the results of Zheng et al. (28) that homozygous mutant mice were obtained at a frequency of 1 in 4, indicating that a normal IL-1β gene is not required for successful embryonic development. The structure of the targeted locus was confirmed by Southern blotting using as the probe a fragment of the IL-1β gene derived from a portion of the 5th intron external to the targeting vector (Fig. 1 *a*). Hybridization with a *neo* probe confirmed that there was only a single integration event (Fig. 1 *b*). Again confirming the results of Zheng et al. (28), IL-1β–deficient mice displayed a grossly normal phenotype, demonstrating that IL-1β is not essential for postnatal development.

The development of cells of the hematopoietic lineage was assessed by flow cytometry and histological analysis. IL-1β–deficient and wild-type mice did not differ significantly in the numbers of peripheral blood erythrocytes, lymphocytes, monocytes, neutrophils, or platelets (data not shown). Similarly, the cell content and architecture of the spleen and thymus were grossly similar in IL-1β–deficient and wild-type mice (data not shown).

Evaluation of IL-1 Expression. Functional ablation of the IL-1β gene was confirmed by Northern blotting, immunoprecipitation, and ELISA. PEC were elicited by i.p. injection of thioglycollate. 3 d after thioglycollate injection, exudate cells were harvested by peritoneal lavage. Similar numbers of macrophages were recovered from the wild-type and the IL-1β–deficient mice, demonstrating that macrophage recruitment to the peritoneal cavity can occur in the absence of this cytokine.

PEC from wild-type, heterozygous, and IL-1β–deficient mice were incubated for 4 h in the presence or absence of LPS. By Northern blotting, there was no detectable IL-1β mRNA in LPS stimulated PEC from the IL-1β–deficient mice (Fig. 1 *c*). IL-1β mRNA was present at an intermediate level in LPS-stimulated PEC from mice heterozygous for IL-1β deficiency. Given that the mutant IL-1β gene carried alterations limited to exon 4, and that this exon is not known to affect expression of the IL-1β gene, we speculate that the absence of detectable IL-1β transcripts in IL-1β–deficient mice represents dramatic destabilization of the IL-1β mRNA rather than obliteration of IL-1β transcription. Hybridization with an IL-1α cDNA probe showed indistinguishable induced levels of LPS-induced IL-1α transcripts in wild-type, heterozygous, and IL-1β–deficient mice Fig. 1 *c*). This confirmed that PEC from IL-1β–deficient mice retained responsiveness to LPS and that LPS-induced expression of IL-1α was not dependent on expression of IL-1β.

Analysis of IL-1 biosynthesis by pulse-chase labeling and immunoprecipitation confirmed that IL-1β protein was undetectable in mice homozygous for IL-1β deficiency (Fig. 2 *a*). In contrast, biosynthesis of pro-IL-1α was indistinguishable in wild-type and IL-1β–deficient mice (Fig. 2 *b*). In normal LPS-stimulated macrophages, induction of cellular apoptosis by treatment with 5 mM ATP is known to trigger efficient maturation of both IL-1 isoforms and their release from the cell (41). Consequently, we treated LPS-stimulated PEC from wild-type and IL-1β–deficient mice with ATP and evaluated the processing and release of IL-1α (Fig. 2, *a* and *b*). The ATP-induced release of mature IL-1α from wild-type and IL-1β–deficient cells was also indistinguishable. This demonstrated that release of IL-1α was not dependent on IL-1β. This is in contrast to data obtained in ICE-deficient mice in which in addition to the expected

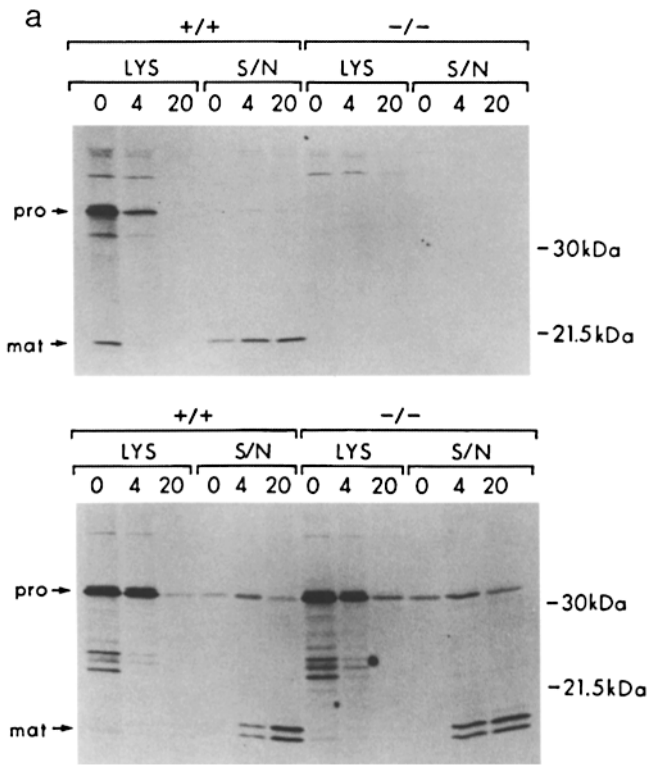

Figure 2. Following stimulation with LPS for 2 h, thioglycollate-elicited PEC were pulse labeled with ^{35}S-methionine for 30 min, then treated with 5 mM ATP for 20 min. Supernatants and lysates were harvested at the indicated times of chase and immunoprecipitated with (*a*) anti-IL-1β or (*b*) anti-IL-1α monoclonal antibodies.

deficiency of cellular processing and release of IL-1β there was an unexpected reduction in the release of IL-1α (42).

Finally, IL-1 levels in the supernatants of LPS-stimulated, ATP-treated PEC were measured by ELISA (Table 1). In the absence of ATP, neither IL-1α nor IL-1β was detected in the supernatants of wild-type PEC. Incubation with 5 mM ATP for 4 h lead to the release from these cells of 1,082 pg/ml of IL-1α and 783 pg/ml of IL-1β. No detectable IL-1β was released from IL-1β–deficient cells. In contrast, IL-1β–deficient cells released 1,264 pg/ml of IL-1α following treatment with LPS and ATP.

Thus, as assessed by Northern blotting, pulse-chase labeling and immunoprecipitation, and ELISA, gene targeting resulted in complete functional ablation of the IL-1β locus. All of the data suggested that normal expression and cellular release of IL-1α were retained. This distinguishes the biochemical characteristics of the IL-1β–deficient strains from ICE-deficient strains in which IL-1α biosynthesis is unaffected, but the release of IL-1α from activated cells is substantially reduced (42, 43).

Unaltered Sensitivity to Endotoxic Shock in IL-1β–deficient Mice. Treatment of normal mice with high doses of endotoxin results in the production and release of several proinflammatory cytokines, including TNF, IL-1, IL-6, and IFN-γ. These, in turn, appear to be the major mediators of

Table 1. *IL-1 Release from Cultured Peritoneal Macrophages*

	IL-1α	IL-1β
	pg/ml	
Wild-type		
Control	<15	<10
LPS	<15	<10
LPS/ATP	1,082 ± 35	783 ± 11
IL-1β–deficient		
Control	<5	<10
LPS	106 ± 1	<10
LPS/ATP	1,265 ± 19	<10

1 × 10^6 PEC were allowed to adhere overnight in a 12-well dish and treated with 1 μg/ml LPS for 2 h. The cells were then washed and incubated either with ATP (5 mM) or with medium alone for 4 h. Then supernatants were collected and assayed in triplicate by ELISA. Data presented are average levels of IL-1 ± SEM. Limits of detection for IL-1α and IL-1β were 15 pg/ml and 10 pg/ml, respectively.

the lethal effects of endotoxin (44). Blocking the action of these cytokines reduces endotoxin-induced mortality as evidenced by endotoxin resistance in TNF receptor type 1-deficient (45), IFN-γ receptor-deficient (46), and ICE-deficient mice (42). In addition, administration of the IL-1ra to both mice (47) and rabbits (48) protects from the lethal effects of endotoxic shock.

The sensitivity of IL-1β–deficient and wild-type mice to endotoxin was determined both with pretreatment with D-galactosamine (data not shown) and without galactosamine (Fig. 3). Death occurred with a similar time course in the wild-type and the IL-1β–deficient animals. The sensitivity of IL-1β–deficient mice to endotoxin-induced death is of

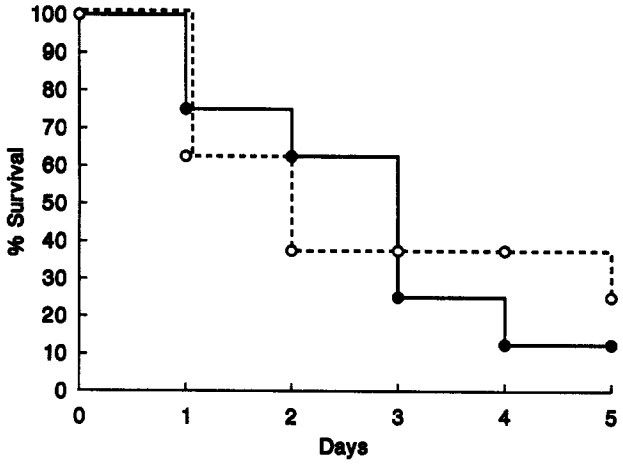

Figure 3. Susceptibility to endotoxic shock. Groups of eight IL-1β$^{-/-}$ (*open circles*) and eight wild-type (*closed circles*) mice received 1.5 mg *E. coli* LPS (0127:B8) in sterile PBS by i.p. injection.

interest in light of the defined resistance to endotoxin conferred by treatment with the IL-1ra and by inherited deficiency of ICE. As ICE-deficient and IL-1ra–treated mice have defects in function of both IL-1β and IL-1α, whereas IL-1β–deficient mice retain IL-1α expression and release, these data support a significant role for IL-1α in systemic endotoxemia.

IL-1β Is Required for Normal Contact Hypersensitivity Responses. In order to test the role of IL-1β in contact hypersensitivity responses, we examined responsiveness in wild-type and IL-1β–deficient mice after sensitization with varying concentrations of TNCB. Sensitization of wild-type mice with 25 μl of TNCB ranging in concentration from 0.01 to 3.0% lead in a dose-dependent fashion to antigen-specific swelling at the sight of challenge 24 h after subcutaneous injection of 10 mM TNBS into the footpad. Significant footpad swelling was detected after sensitization with 0.03% TNCB, and maximal swelling was observed after sensitization with 0.1% TNCB (Fig. 4). In contrast, mice deficient in IL-1β showed no footpad swelling following sensitization with 0.1% TNCB, requiring 1% TNCB to achieve a response equivalent to the wild-type mice. Histological evaluation of the sites of antigen challenge showed an inflammatory cell infiltrate in wild-type mice sensitized with 0.1% TNCB compared to nonsensitized controls (Fig. 5, *a* and *b*), but no detectable infiltrate in sensitized IL-1β–deficient mice (Fig. 5 *c*). Similarly, when cells were isolated from the axillary and inguinal lymph nodes draining the site of sensitization and incubated with TNBS-haptenated spleen cells, dose-dependent proliferation was detected with cells from wild-type mice sensitized with 0.1% TNCB, whereas no specific proliferation was detected in IL-1β–deficient mice treated with this same concentration of sensitizing antigen (Fig. 6). Lymphocytes from nonsensitized wild-type mice or wild-type mice sensitized with low doses of TNCB (0.01 and 0.03%) did not proliferate in response to TNBS-haptenated spleen cells (data not shown). These data suggest, then, that the proliferative response in sensitized wild-type mice represented T cell priming in vivo. Together, these data all demonstrate a significant defect in the ability of IL-1β–deficient mice to manifest a contact hypersensitivity reaction.

Zheng et al. previously reported that in an independently derived IL-1β–deficient mouse strain the contact hypersensitivity response to oxazolone was normal (28). We tested the response to topical oxazolone in the IL-1β–deficient strain described here. Wild-type and IL-1β–deficient mice were sensitized by topical application of oxazolone (100 ml of 0.01%, 0.04%, 0.1%, 0.4%, 1%, and 4%) to the shaved abdomen and 5 d later sensitivity was tested by application of 50 μl of 1% oxazolone to the ear. After an additional 24 h, the degrees of ear swelling were indistinguishable in wild-type and IL-1β–deficient mice (data not shown). These data are in dramatic contrast to results using TNCB and suggest that the cutaneous responses to these two antigens are not equivalent. Although the mechanism remains undefined, differences in the methods to elicit the hypersensitivity response may underlie the differences in the TNCB and the oxazolone responses. For example, we measured hypersensitivity to TNCB following injection of soluble TNBS into the footpad, whereas hypersensitivity to oxazolone was measured following topical application to the ear. Thus, the mechanism by which eliciting antigen is detected in the footpad and the ear may not be identical. Additionally, TNCB was applied at the time of sensitization as a solution of 25% olive oil in acetone, whereas oxazolone was applied dissolved in acetone alone. Thus, the local handling of the sensitizing antigen may be different for the two antigens. Finally, it remains possible that the cellular constituents responsible for TNCB and oxazolone reactivity are not the same. Regardless, the data presented here demonstrate a dramatic requirement for IL-1β in the response to TNCB.

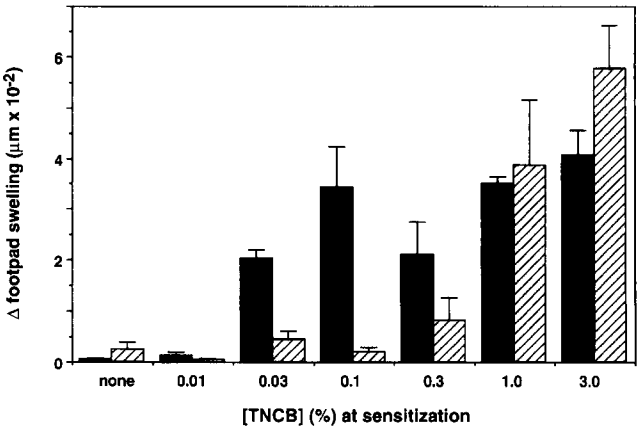

Figure 4. Reduced contact hypersensitivity response in IL-1β$^{-/-}$ mice. 8–12-week-old IL-1β–deficient mice and their wild-type littermates were painted on their shaved abdomens with the indicated concentrations of TNCB in 25 μl olive oil/acetone (1:3). 5 d later, the mice were challenged by injection of 33 ml of 10 mM TNBS into the right footpad. PBS was injected into the left footpad. After 24 h, footpad swelling was measured. Solid bars, wild-type mice; hatched bars, IL-1β–deficient mice.

To investigate at which stage of the TNCB response IL-1β exerted its action, we injected purified recombinant murine 17 kD IL-1β (rIL-1β) (49) intradermally immediately before sensitization at the site where the sensitizing antigen was to be applied. Pretreatment with rIL-1β but not with saline resulted in dose-dependent restoration of responsiveness to antigen challenge five days later (Fig. 7) and a restoration of the inflammatory cell infiltrate examined histologically (Fig. 5 *d*). These data support a role for IL-1β specifically during the sensitization phase of the contact hypersensitivity response. Sensitization of the IL-1β–deficient mice is, however, demonstrated when high concentrations of antigen are applied to the skin. We speculate that this indicates that IL-1β acts on or through cells whose role is to enhance antigen uptake, processing, or delivery to the antigen-responding cells and that high doses of antigen bypass these functions.

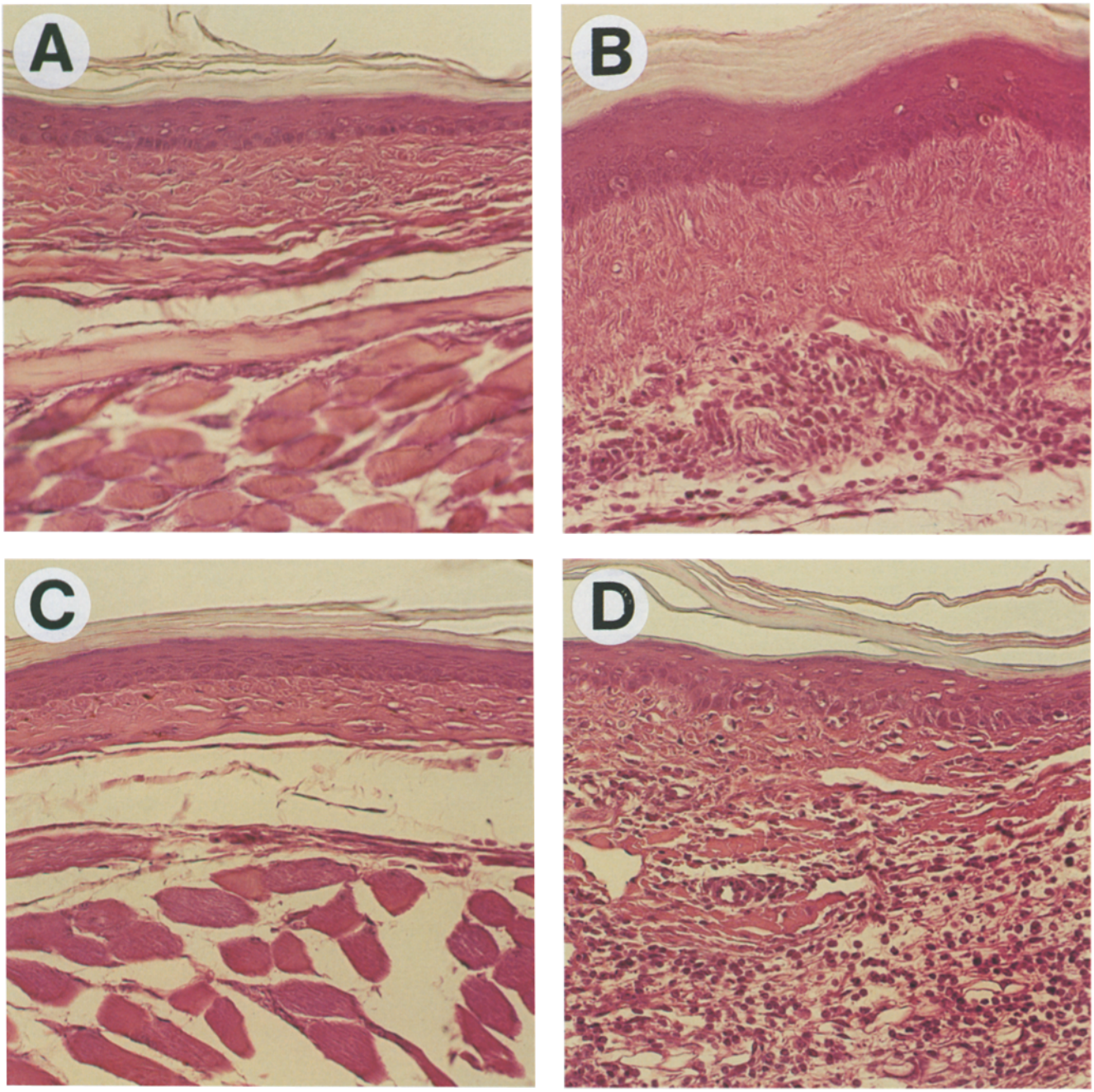

Figure 5. Inflammatory infiltrates in sections of footpad of TNBS-challenged mice. Mice sensitized by topical application of 0.1% TNCB or vehicle alone were challenged with 10 mM TNBS injected into the footpad. Tissue was harvested 24 h later, fixed in 10% neutral buffered formalin, and sections stained with hematoxylin and eosin. (*a*) Wild-type mice mock-sensitized with vehicle alone; (*b*) wild-type mice sensitized with TNCB; (*c*) IL-1β–deficient mice sensitized with TNCB; (*d*) IL-1β–deficient mice treated intradermally with 50 ng recombinant IL-1β at the sensitization site immediately before application of TNCB.

Both Langerhans cells and keratinocytes have been shown to express IL-1β in the epidermis (26, 27). Using mononuclear phagocytes, we have demonstrated that induction of cellular apoptosis in vitro leads to rapid release of bioactive IL-1β (41). Our current data suggest either that these Langerhans cells or keratinocytes possess a novel mechanism for IL-1β release that is expressed in situ in the skin, but not in vitro, or that apoptosis of the IL-1β–producing cell is a characteristic of contact hypersensitivity responses. Alternatively, they may imply the presence of a novel IL-1 receptor in skin that can interact with unprocessed 31 kD pro–IL-1β as an activating ligand, or that may have specificity for IL-1β, perhaps not recognizing IL-1α. A precedent for a receptor that can discriminate IL-1β from IL-1α

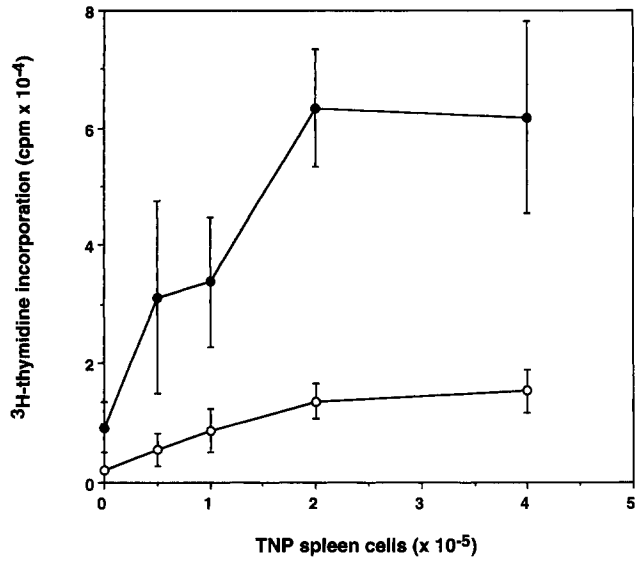

Figure 6. Absent proliferative response to contact antigen in IL-1β–deficient mice. Lymphocytes were harvested from axillary and inguinal lymph nodes six days after sensitization with TNCB. Proliferative responses to mitomycin-C–treated TNP-haptenated sv129 spleen cells were measured as described in Methods. Data represent average ± SEM of triplicate determinations. Closed circles, wild-type mice; open circles, IL-1β–deficient mice.

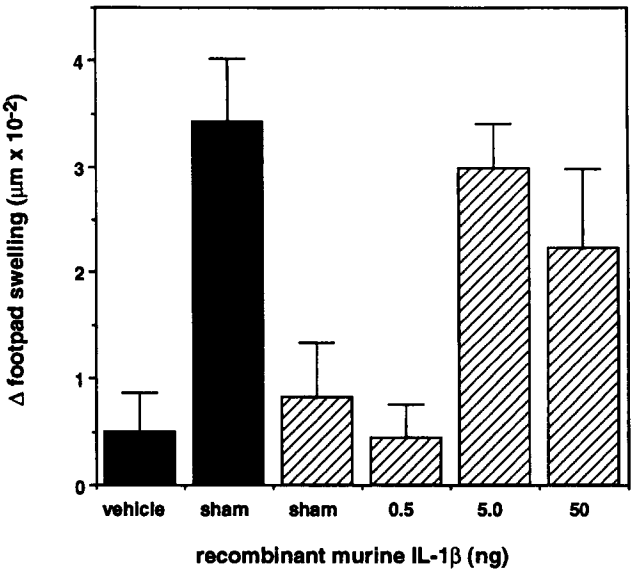

Figure 7. Contact hypersensitivity can be restored by intradermal rIL-1β. The indicated amounts of recombinant murine IL-1β were injected intradermally at the sites of sensitization immediately before the application of 25 ml of 0.1% TNCB. Challenge and measurement of footpad swelling were as described in Fig. 4. Closed bars, wild-type mice; hatched bars, IL-1β–deficient mice.

comes from vaccinia virus where a secreted IL-1 receptor with specificity for the IL-1β isoform has been reported (50).

Our observation that IL-1β plays a dramatic role in contact hypersensitivity responses to TNCB at low to moderate doses of sensitizing antigen caution against excluding an activity of this or any cytokine in a physiological response by experiments using high levels of stimuli. Although we have not defined the mechanism by which high doses of sensitizing antigen overcome the defect in the contact hypersensitivity response in IL-1β–deficient mice, the use of low doses of sensitizing antigen may uncover a role for IL-1β in lowering the threshold for responsiveness. Conversely, high doses of sensitizing antigen may circumvent the fundamental character of the response being analyzed.

In summary, we report the generation of a mouse strain rendered deficient in expression of IL-1β by gene targeting. This strain confirms that IL-1β is not required for viable embryonic development or normal post-natal growth. Analysis of this strain discloses no evidence of an obligatory role for IL-1β in lymphoid cell development or in the development of other bone-marrow derived cells. Additionally, absence of IL-1β does not alter the sensitivity to endotoxin-induced death in two models of endotoxic shock. Deficiency of IL-1β does, however, result in a defect in contact hypersensitivity responsiveness to TNCB, with this effect expressed at the level of sensitization. Additional evaluation of this strain will permit definition of the cellular elements in the epidermis that mediate this effect. It will also permit definition of whether IL-1β acts selectively in the epidermis, or generally in sensitization at all epithelial surfaces, particularly in the respiratory and gastrointestinal tracts. As such, this IL-1β–deficient mouse strain may prove valuable in identifying new targets with clinical relevance to human hypersensitivity disease.

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