In Vivo Administration of Interleukin 1 to Normal Mice Depresses Their Capacity to Elicit Contact Hypersensitivity Responses: Prostaglandins Are Involved in This Modification of Immune Function

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The administration of pyrogenic doses of interleukin 1 (IL-1) to normal mice before contact sensitization with dinitrofluorobenzene (DNFB) resulted in a significant reduction in the intensity of the elicited contact hypersensitivity (CH) responses. Adoptive transfer experiments established no difference between normal and IL-1-pretreated mice regarding their capacity to generate splenic suppressor-cell activity and lymph node effector-cell activity in response to DNFB. However, a marked reduction in the intensity of elicited responses was observed when primed CH-effector cells, obtained from normal donors, were adoptively transferred to IL-1-pretreated recipients. This finding was paralleled by a consistent reduction in the ability of the adoptively transferred cells to infiltrate the tissue sites of antigen challenge in the IL-1-pretreated animals. Treatment of mice with indomethacin, a potent inhibitor of prostaglandin production, abrogated the capacity of IL-1 to depress CH responses following skin sensitization with DNFB. Similarly, indomethacin was also capable of abrogating the ability of IL-1 to depress CH responses of adoptive recipients of primed CH-effector cells. Our results indicate that the capacity of IL-1 to depress CH responses in normal mice is due to an indomethacin-sensitive process, presumably mediated through the IL-1-induced generation and action of prostaglandins. This was supported by our finding that treatment of mice with arachidonic acid or prostaglandin E_2 caused a similar type of inhibition. The mechanism(s) responsible for this effect appears to act at the efferent level of the CH response, as evidenced by the reduced capacity of CH-effector cells to infiltrate the tissue sites of antigen challenge. *J Invest Dermatol 88:380–387, 1987*

Exposure of mice to ultraviolet radiation (UVR) causes a depression in their ability to elicit contact hypersensitivity (CH) responses to topically applied skin-reactive chemicals [1,2]. This depression in reactivity occurs not only when the contact sensitizing (CS) agent is applied directly to the irradiated skin site, but also when the hapten is applied to nonirradiated skin sites [3–5]. The latter phenomenon has been referred to as a systemic depression of CH responses and requires large doses of UVR for elicitation [4,5]. A similar type of systemic depression in immune responsiveness that has recently been described occurs when the inflammatory agent lipopolysaccharide (LPS) is administered to animals before the topical application of skin-reactive chemicals [6]. Lipopolysaccharides and UVR are potent inducers of inflammatory responses in vivo and are both capable of inducing the production of the multifunctional hormone, interleukin 1 (IL-1) [6–11].

Since both UVR and LPS stimulate IL-1 production in vivo, the question was raised as to whether IL-1 itself had the capacity to depress CH responses in vivo. Our preliminary results [6] have demonstrated that the i.v. administration of pyrogenic doses of IL-1 to normal mice depressed a subsequent immunologic response to the CS agent dinitrofluorobenzene (DNFB). In this report we describe the results of experiments that were designed to elucidate the mechanism(s) by which IL-1 depresses the intensity of CH responses to CS agents.

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Abbreviations:
CH: contact hypersensitivity
CS: contact sensitizing
DNFB: dinitrofluorobenzene
ETAF: epidermal cell-derived thymocyte activating factor
FCS: fetal calf serum
IL-1: interleukin 1
LPS: lipopolysaccharide
PMN: polymorphonuclear leukocyte
SAP: serum amyloid-P
scIL-1: stratum corneum-derived ETAF/IL-1
UVR: ultraviolet radiation

MATERIALS AND METHODS

Experimental Animals C3H/HeN-strain mice of both sexes were obtained from the Animal Production Facility, National Institutes of Health. All mice were housed at a maximum density of 5 animals per 7 × 11 inch cage and maintained on Wayne Sterilizable Lab Blox and acidified water ad libitum. Animals were sex matched and 8–12 weeks old at the onset of any given ex-

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periment. Four to five animals were employed within each experimental group. Most experiments were repeated at least twice with similar results.

Sources of Epidermal Cell-Derived Thymocyte Activating Factor (ETAF)/IL-1 Purified human IL-1 purchased from Genzyme (Boston, Massachusetts) was used as a standard to quantitate the activity of ETAF/IL-1 used in these studies. This material is provided by the manufacturer, in a form stabilized with fetal calf serum (FCS), and is therefore unsuitable for in vivo studies. The ETAF/IL-1 employed in most of the in vivo studies reported herein was prepared from human heel callus stratum corneum and semipurified as described previously [12]. Stratum corneum-derived ETAF/IL-1 (scIL-1) is totally free of any interleukin 2, interleukin 3, and any other colony-stimulating activities (W. E. Samlowski et al, submitted for publication) and provides an easy and inexpensive source of high-specific-activity material for in vivo and in vitro bioassays of ETAF/IL-1 mediated effects. Recombinant human IL-1 was employed in selected in vivo experiments to verify that the effects being observed were not due to a minor contaminant in the stratum corneum-derived preparation. This material was generously provided by Dr. Robert Newton (duPont de Nemours & Company, Glenolden, Pennsylvania).

Prostaglandin and Arachidonic Acid Prostaglandin E_2 was purchased from Upjohn Co. (Kalamazoo, Michigan) and diluted immediately before use to a concentration of 100 μg/ml. Arachidonic acid-containing pellets were obtained from Innovative Research of America (Rockville, Maryland) and are designed to release approximately 375 μg/day over a 20-day period following subcutaneous implantation.

Prostaglandin Inhibitors Pellets containing specified doses of indomethacin were obtained from Innovative Research of America. The pellets were inserted subcutaneously by trocar 24–48 h before initiating an experimental protocol, and have the capacity to slowly release the incorporated drug at a constant rate (1.25–2.5 μg/day) over a 20-day period.

In Vivo Analysis of ETAF/IL-1-Mediated Effects Core body temperature of C3H/HeN mice was determined 20 min post injection of ETAF/IL-1 using a rectal probe and a YSI Digital Telethermometer (Model 49TA, Fisher Scientific Co., Pittsburgh, Pennsylvania). Circulating murine neutrophils were quantitated 24 h post injection of ETAF/IL-1-containing preparations by the removal of 50 μl of blood from each experimental and control mouse in heparinized capillary tubes. The total number of leukocytes was determined with a hemocytometer and differential analysis of leukocytes was performed on Wright's stained blood smears. Plasma obtained from experimental mice 24 h post injection of ETAF/IL-1 was assayed quantitatively for serum amyloid-P (SAP) by radial immunodiffusion employing a rabbit antimurine SAP antibody [7].

Sensitization and Elicitation of Contact Hypersensitivity Normal mice were sensitized by applying 25 μl of 0.25% DNFB (Sigma Chemical Co., St. Louis, Missouri) in a 4:1 acetone:olive oil mixture to the shaved ventral surface on day 0 and day 1. The presence of CH was established by applying 10 μl of the solution to the right ear on day 4. The extent of swelling was used as a measure of CH and is expressed as the difference in thickness between the challenged right and unchallenged left ear as measured with an engineer's micrometer (Mifutoyo, Japan) 24 and 48 h following challenge. The percent depression of CH responses caused by IL-1 administration was calculated from the formula:

$$\% \text{ depression} = 1 - \frac{\text{experimental} - \text{challenge only}}{\text{positive control} - \text{challenge only}} \times 100.$$

Adoptive Transfer of Suppressor Cells Normal mice or mice that had been injected i.v. with a pyrogenic dose of IL-1 24 h previously were sensitized with 25 μl of a 25% DNFB solution in acetone:olive oil (4:1) on the shaved abdomen and 10 μl on each footpad and ear on day 0 and day 1. Four days following sensitization, spleens were surgically excised and gently dissociated in RPMI 1640 (Dutchland Laboratory Animals, Inc., Denver, Pennsylvania) supplemented with 5% FCS (Hy-Clone Sterile Systems, Inc., Logan, Utah). The resulting single cell suspension was transferred i.v. into normal recipient mice (1 $\times$ 10^8 cells/recipient). Recipients were sensitized with 0.25% DNFB on their shaved abdomen 1 h and 24 h following splenocyte injection. Five days following sensitization, all groups of mice were challenged by applying 10 μl of 0.25% DNFB to the right ear. The percent suppression was calculated according to the formula:

$$\% \text{ suppression} = 1 - \frac{\text{adoptive recipient} - \text{challenge only}}{\text{positive control} - \text{challenge only}} \times 100.$$

Adoptive Transfer of Effector Cells Normal mice or mice that had been injected i.v. with IL-1 24 h previously were sensitized with 25 μl of a 0.25% DNFB solution in acetone:olive oil (4:1) on the shaved abdomen and 10 μl on each footpad and ear on day 0 and day 1. Four days following sensitization, inguinal, axillary, and brachial lymph nodes were excised and gently dissociated in RPMI 1640 supplemented with 5% FCS. The resulting single cell suspension was transferred i.v. into normal recipient mice (30 $\times$ 10^6 cells/recipient) that were challenged immediately on the right ear with 10 μl of a 0.25% DNFB solution. Ear swelling was measured 24 and 48 h following challenge.

Effector Cell Localization Assay Normal mice were sensitized with 25 μl of 0.25% DNFB on the shaved abdomen and 10 μl on each footpad and ear on day 0 and day 1. Four days following sensitization, inguinal, axillary, and brachial lymph nodes were excised and gently dissociated in RPMI 1640 supplemented with 5% FCS. The lymphocyte suspension was adjusted to approximately 10^8 cells/ml in RPMI and incubated for 30 min at 37°C in the presence of 25–50 μCi of sodium chromate (^{51}Cr, sp act 50–400 mCi/mg Cr, Amersham, Arlington Heights, Illinois). Immediately following incubation, the cells were washed 3 times with RPMI 1640 medium to remove unbound radiolabel. The resulting lymphocyte mixture was transferred i.v. into appropriate recipient mice that were challenged immediately on the ear with 10 μl of a 0.25% DNFB solution. Ear swelling was measured 24 h following challenge. Immediately following ear thickness measurements, the mice were sacrificed and both ears were surgically excised and assayed on a Beckman gamma counter for the amount of ^{51}Cr localized to the DNFB-challenged and unchallenged ears.

RESULTS

Systemic Administration of IL-1 to Mice Reduces Their Ability to Elicit a CH Response The exposure of normal mice to UVR or LPS before contact sensitization causes a reduction in their capacity to elicit CH responses [3–6]. This observation led us to question whether the immunodepressive activities of UVR and LPS might be mediated through a common pathway, possibly through the production of IL-1 or one of the many known bioactivities induced by this inflammatory mediator. To analyze this possibility, the effects of IL-1 in vivo on the generation of CH responses were determined. Groups of normal mice were injected i.v. with pyrogenic doses (100, 50, or 25 units) of human scIL-1. These doses of scIL-1 cause an increase in core body temperature, stimulate the release of neutrophils from bone marrow stores, and stimulate liver hepatocytes to produce acute phase proteins (Table I). Twenty-four hours following the administration of scIL-1, the experimental groups and a group of control animals were contact sensitized by topical application to the abdomen of DNFB. Five days after skin sensitization all animals were challenged by application of the same hapten to the ear. The results of this experiment (Table II) demonstrate that the i.v. injection of scIL-1 to normal mice 24 h before skin sensitization

Table I. In Vivo Administration of scIL-1 to Mice Induces an Acute Phase Response

Experiment #	50 Units Interleukin 1[a]	Δ Temp[b] (°C)	Δ SAP[c] (μg/ml)	Δ PMN[d] (× 10^{-6} cells/ml)
1	+	1.4 ± 0.1	53 ± 5	3.9 ± 0.08
2	+	1.3 ± 0.1	71 ± 2	4.4 ± 0.34

[a]Mice received an i.v. injection of 50 units of scIL-1. The bioactivity of our preparation was compared with a purified preparation of IL-1 obtained from Genzyme.
[b]Core body temperature was analyzed 20 min post injection. The normal range of core body temperature in these experiments was 36.7°C ± 0.2 and 36.6°C ± 0.2, respectively.
[c]Peripheral blood samples were obtained from all test animals 24 h post injection and plasma samples were analyzed for serum amyloid-P (SAP) levels by radial immunodiffusion. SAP values obtained from normal mice for both experiments were 60 ± 3 μg/ml and 49 ± 1 μg/ml.
[d]PMN were quantitated from 24-h postinjection peripheral blood samples. Normal values were 1.78 ± 0.18 × 10^6 cells/ml and 1.8 ± 0.3 × 10^6 cells/ml.

with DNFB markedly reduces their ability to elicit a CH response in a dose-dependent manner. Further, the exposure of a similar preparation of scIL-1 to heat (90°C for 30 min) abrogated its capacity to inhibit the development of a CH response. This experiment has been repeated several times using both semipurified human scIL-1 [12] and recombinant human IL-1, with identical results (data not shown).

Studies of Effector and Suppressor Cell Activity in IL-1-Pretreated Mice Contact hypersensitivity is a cell-mediated immune response that associates with both the generation of effector cells, for elicitation, and the generation of suppressor cells that are believed to regulate the intensity and duration of this response. We therefore questioned whether the scIL-1-treated animals differed from normals in their capacity to generate either suppressor or CH-effector cells in response to contact sensitization.

To assess suppressor cell generation, groups of normal and scIL-1-pretreated mice were contact sensitized with DNFB. Five days later these animals were sacrificed and their spleens were harvested and dissociated in vitro to obtain a single cell suspension. Splenocytes (1 × 10^8) from both donor groups were then injected i.v. into groups of naive recipients that were immediately sensitized with DNFB. All animals were then ear-challenged with DNFB 5 days later. The results of this study (Table III*A*) establish that following DNFB sensitization, significant suppressor cell activity could be found in the spleens obtained from both normal and scIL-1-pretreated donors. The adoptive transfer of splenocytes from nonsensitized animals into normal recipients, that were subsequently sensitized with DNFB and challenged, had minimal effect on the generation of CH responses. This finding is consistent with the observations made by others indicating that both UVR-exposed and normal animals exhibit systemic suppressor-cell activity subsequent to contact sensitization to topically applied haptens [13]. An additional experiment, where serum from scIL-1-pretreated and DNFB-sensitized donors (0.3 ml) was adoptively transferred into normal recipients, was found to have no suppressive effect (data not shown).

In a parallel study we analyzed whether CH-effector cells were induced in scIL-1-pretreated animals subsequent to contact sensitization. Groups of normal and IL-1-pretreated animals were contact sensitized with DNFB and 5 days later their peripheral lymph nodes were harvested and teased into single cell suspensions. These lymph node cells (30 × 10^6) were injected i.v. into groups of naive recipients, which were immediately ear-challenged with DNFB. Changes in ear thickness were measured 24 h following the hapten challenge. The results (Table III*B*) determined that an equivalent degree of effector-cell activity was present in both the normal and the scIL-1-pretreated animals that were sensitized with DNFB. Therefore, it appears that scIL-1-pretreated animals elicit a depressed CH response despite the presence of both suppressor and effector cell activity that is functionally equivalent to that found in normal animals.

Interleukin 1 Functions by Inhibiting the Effector Arm of the CH Response We next questioned whether the effects of scIL-1 on the generation of CH responses might be associated with a depressed capacity of these animals to mobilize immune effector cells to the peripheral sites of antigen challenge. Groups of normal and scIL-1-pretreated animals (day −1) received 30 × 10^6 DNFB-sensitized effector cells obtained from the peripheral lymph nodes of normal DNFB-primed donors. Immediately following lymphocyte injection, all animals were ear-challenged with DNFB. Changes in ear thickness were measured 24 h later. The scIL-1-pretreated recipients were found to exhibit a marked reduction in their capacity to elicit a CH response following the adoptive transfer of DNFB-primed lymph node cells (Table IV, Experiment 1). The degree of inhibition in the capacity of scIL-1-treated animals to elicit a CH response following the adoptive transfer of DNFB-primed lymph node cells was most pronounced when the CH-effector cells were given 1 day following scIL-1 treatment (Table IV, Experiment 2). The scIL-1-treated animals appeared to recover their ability to elicit a CH response by 1–2 weeks post scIL-1 treatment.

The results of our experiments suggested that the effects of IL-1 on CH was due to its capacity to interfere with the effector arm of this immunologic response. To determine whether the adop-

Table II. Capacity of scIL-1 to Inhibit the Ability of Mice to Generate Contact Hypersensitivity Responses Is Dose Dependent

Group #	Dose of scIL-1[a]	DNFB Sensitize[b]	DNFB Challenge[b]	Δ Ear Swelling[c]	Percent Depression[d]
1	—	—	+	1 ± 0.5	—
2	—	+	+	102 ± 2.0	0
3	100 units	+	+	61 ± 2.0	41
4	50 units	+	+	59 ± 3.0	43
5	25 units	+	+	95 ± 2.0	7
6	Δ 100 units[e]	+	+	108 ± 3.0	0

[a]Twenty-four hours before DNFB sensitization mice received an i.v. injection of appropriate doses of scIL-1. The bioactivity of our preparation was compared with a purified preparation of IL-1 obtained from Genzyme.
[b]Mice were sensitized to DNFB by applying 25 μl of a 0.25% solution of DNFB in olive oil (4:1) on their ventral surface. Five days following sensitization these animals were ear-challenged with 10 μl of 0.25% DNFB.
[c]Twenty-four hours after challenge, ear swelling (1 × 10^{-4} inches ± SEM) was measured with an engineer's micrometer. Results are expressed as the thickness of the challenged ear minus the thickness of the unchallenged ear.
[d]Percent depression was calculated using the following equation:

$$\% \text{ depression} = 1 - \frac{\text{swelling (experimental)} - \text{swelling (negative control)}}{\text{swelling (positive control)} - \text{swelling (negative control)}} \times 100.$$

[e]The scIL-1 was heat-inactivated (90°C for 30 min) before i.v. injection.

Table III. Normal Suppressor and Effector Cells to DNFB Are Generated in scIL-1 Pretreated Animals

A. Splenocytes from Normal and scIL-1-Pretreated Mice Demonstrate Equivalent Suppressor Cell Activity in Response to DNFB

Group #	Treatment of Recipient Mice[a]	Ear Swelling[b]	Percent of Normal Response[c]
1	Challenge only	4 ± 2	—
2	Sensitize plus challenge	79 ± 2	100
3	Adoptive transfer of spleen cells from DNFB-sensitized donors; sensitize plus challenge	37 ± 2	44
4	Adoptive transfer of spleen cells from DNFB-sensitized and scIL-1-treated donors; sensitize plus challenge	35 ± 1	41
5	Adoptive transfer of spleen cells from untreated donors; sensitize plus challenge	67 ± 0	84

B. scIL-1-Pretreated Mice Elicit Normal Effector Cell Response Following Skin Sensitization With DNFB

Group #	Treatment of Recipient Mice[d]	Ear Swelling[b]	
		Experiment #1	Experiment #2
1	Challenge only	3 ± 1	2 ± 1
2	Adoptive transfer of lymph node cells from DNFB-sensitized donors; plus challenge	32 ± 4	29 ± 1
3	Adoptive transfer of lymph node cells from DNFB-sensitized and scIL-1-treated donors; plus challenge	39 ± 3 (0)[e]	30 ± 1 (0)

[a]Recipient mice received an i.v. injection of normal splenocytes (1×10^8) or DNFB-sensitized splenocytes (1×10^8) elicited in normal or scIL-1-pretreated (50 units) donor mice as described in *Materials and Methods*. One hour and 24 hours following injections, recipient mice were sensitized on their stomach with a 0.25% DNFB solution in acetone:olive oil (4:1). Five days following sensitization, mice were ear-challenged with the same antigen.

[b]Twenty-four hours following challenge, ear swelling (1×10^{-4} inches ± SEM) was measured with an engineer's micrometer. Results are expressed as the thickness of the challenged ear minus the thickness of the unchallenged ear.

[c]The capacity to elicit a CH response was calculated as a percentage of the positive control.

[d]Recipient mice received an i.v. injection of DNFB-sensitized lymphocytes (30×10^6) elicited in normal or scIL-1-pretreated (50 units) donor mice as described in *Materials and Methods*. Immediately following lymphocyte injection, recipient mice were ear-challenged with a 0.25% DNFB solution in acetone:olive oil (4:1).

[e]Percent deviation from effector response elicited by normal donors.

tively transferred effector cells gain access to the tissue site of antigenic challenge, lymph node cells were obtained from DNFB-primed donors and radiolabeled by incubation with ^{51}Cr before their transfer to normal or scIL-1-pretreated recipients. All animals were subsequently ear-challenged with DNFB. Twenty-four hours following challenge, both the extent of ear swelling, and the amount of radioactivity within each ear was quantitated by surgically excising the ears and counting in a gamma counter.

The results of 3 different experiments (Table V) verified that the IL-1-pretreated animals demonstrate a markedly depressed CH response. This condition was paralleled by a significant reduction in the number of adoptively transferred cells capable of infiltrating the locally induced inflammatory lesion. The results of these experiments indicate that treatment of animals with scIL-1 alters the movement of immune effector cells into tissue sites of antigen deposition.

Table IV. Pretreatment of Normal Animals With scIL-1 Inhibits the Ability of Adoptively Transferred, Antigen-Sensitized Lymph Node Cells to Mediate Contact Hypersensitivity Responses

Experiment #	Treatment of Recipients			Δ Ear Swelling[d]	Percent Depression[e]
	Adoptive Transfer of DNFB-Sensitized Lymphocytes[a]	scIL-1[b]	Challenge[c]		
1	—	—	+	3 ± 2	—
	+	—	+	34 ± 4	0
	+	(day-1)	+	13 ± 2	68
2	—	—	+	3 ± 2	—
	+	—	+	32 ± 1	0
	+	(day-1)	+	7 ± 1	86
	+	(day-4)	+	12 ± 1	69
	+	(day-7)	+	22 ± 1	36
	+	(day-14)	+	24 ± 1	28

[a]DNFB-sensitized lymphocyte populations from animals prepared as adoptive transfer donors as described in *Materials and Methods*. Each recipient animal received 30×10^6 cells.

[b]Recipient mice received an i.v. injection of 50 units IL-1 at times specified before adoptive transfer procedure.

[c]Recipient mice were ear-challenged with 10 μl of a 0.25% solution of DNFB in acetone:olive oil (4:1) immediately following adoptive transfer.

[d]Twenty-four hours following challenge, ear swelling (1×10^{-4} inches ± SEM) was measured with an engineer's micrometer. Results are expressed as the thickness of challenged ear minus the thickness of unchallenged ear.

[e]Percent depression was determined using the following equation:

$$\% \text{ depression} = 1 - \frac{\text{swelling (experimental)} - \text{swelling (negative control)}}{\text{swelling (positive control)} - \text{swelling (negative control)}} \times 100.$$

Table V. Pretreatment of Animals With scIL-1 Results in a Reduced Capacity of DNFB-Sensitized Effector Cells to Enter Tissue Sites of Antigen Challenge

	Treatment of Recipients				
Experiment #	Adoptive Transfer of DNFB Sensitized Lymphocytes[a]	scIL-1[b]	Challenge[c]	Δ Ear Swelling[d]	Δ CPM in Challenged Ear[e]
1	—	—	+	2 ± 1	—
	+	—	+	35 ± 3 (0)[f]	1054 ± 102 (0)
	+	+	+	13 ± 1 (67)	218 ± 41 (79)
2	—	—	+	5 ± 1	—
	+	—	+	38 ± 1 (0)	285 ± 3 (0)
	+	+	+	13 ± 1 (76)	100 ± 17 (65)
3	—	—	+	6 ± 2	—
	+	—	+	36 ± 1 (0)	1217 ± 129 (0)
	+	+	+	15 ± 1 (70)	776 ± 89 (36)

[a]DNFB-sensitized lymphocyte populations from animals prepared as adoptive transfer donors were labeled with ^{51}Cr as described in *Materials and Methods*. Each recipient animal received 30×10^6 cells.
[b]Recipient mice received an i.v. injection of 50 units scIL-1 24 h before adoptive transfer procedure.
[c]Recipient mice were ear-challenged with 10 μl of a 0.25% solution of DNFB in acetone:olive oil (4:1) immediately following adoptive transfer.
[d]Twenty-four hours following challenge, ear swelling (1×10^{-4} inches ± SEM) was measured with an engineer's micrometer. Results are expressed as the thickness of the challenged ear minus the thickness of the unchallenged ear.
[e]Following ear measurements, mice were sacrificed and both ears were removed and analyzed for amount of radioactivity. Results are expressed as the cpm in challenged ear minus cpm in unchallenged ear.
[f]Numbers in parenthesis represent the percentage of depression.

The Capacity of scIL-1 to Inhibit CH Responses Appears to Be Mediated Through the Action of Prostaglandins We questioned whether the stimulation of prostaglandin production by scIL-1 was involved in the scIL-1-induced depression of CH responses. We first chose to analyze this possibility by using the drug indomethacin, a known inhibitor of the cyclooxygenase pathway and therefore prostaglandin synthesis. Biodegradable pellets that release approximately 2.5 μg/day of indomethacin in vivo on a continuous basis were implanted subcutaneously into groups of mice 48 h before the administration of 50 units of scIL-1. Twenty minutes after scIL-1 treatment core body temperature was determined with a rectal thermometer, and at 24 h blood from these animals was analyzed for changes in the concentration of SAP and the number of circulating neutrophils. The data presented in Table VI determined that treatment of animals with this small dose of indomethacin caused only a slight reduction in the scIL-1-induced fever. However, the IL-1-induced synthesis of acute phase proteins and the release of polymorphonuclear leukocytes (PMNs) by the bone marrow were markedly reduced in animals receiving this type of indomethacin therapy. Further experiments, employing far larger doses of indomethacin (100 g) given i.p. at the same time as the scIL-1, were able to demonstrate that this drug was also capable of abrogating the pyrogenic activity of this cytokine (data not shown). A daily dose of indomethacin of 2.5 μg was used in our experiments to determine the role of prostaglandins on the immunomodulatory activity of scIL-1. Fifty units of scIL-1 were injected i.v. into normal mice or mice that had previously been given a subcutaneous implant of an indomethacin-containing pellet. Twenty-four hours following the scIL-1 injection, both groups of animals, along with normal controls, were contact sensitized by topical application of DNFB. Five days after skin sensitization all animals were ear-challenged with the same hapten. The results presented in Table VII demonstrate that indomethacin treatment abrogates the capacity of IL-1 to depress the development of CH responses. An additional experiment established that the adoptive transfer of lymph node cells from DNFB-sensitized donors to IL-1-pretreated recipients (either scIL-1 or human recombinant $IL\text{-}1_\beta$) allowed a normal CH response if the animals had also received an indomethacin-containing pellet (Table VIII). This result indicates that treatment of animals with indomethacin abrogates the effects of IL-1 on the recipients of antigen-primed effector cells.

Treatment of Mice with Arachidonic Acid or Prostaglandin E_2 Inhibits the Capacity of Normal Recipients to Elicit CH Responses To further support the inhibitory role played by prostaglandins in the effectuation of CH responses subsequent to IL-1 treatment, the following experiment was conducted. Groups of normal mice were pretreated with either recombinant human $IL\text{-}1_\beta$, arachidonic acid (375 μg/day), or PGE_2 (10 μg/day) before the adoptive transfer of DNFB-sensitized lymph node lymphocytes. One half of the animals within each experimental group also received an indomethacin pellet (2.5 μg day). All animals were subsequently ear-challenged with DNFB and the magnitude of ear swelling measured 24 h later. The results of this experiment (Table IX) clearly established that IL-1, arachidonic acid, and PGE_2 were equally effective at inhibiting the capacity of the adoptive recipients to elicit normal CH response. Indomethacin treatment was capable of abrogating the IL-1- and arachidonic acid-induced inhibition, but had no effect on the depression in CH responses caused by PGE_2 itself. This finding adds support to the hypothesis that IL-1 exerts its modulatory activity on CH responses through its capacity to stimulate prostaglandin biosynthesis.

Table VI. Effects of Indomethacin on scIL-1-Induced Acute Phase Response

Group #	Treatment[a]	Δ Temp[b] (°C)	Δ SAP[c] (μg/ml)	Δ PMN[d] ($\times 10^{-6}$ cells/ml)
1	scIL-1	1.6 ± 0.1	53 ± 5	3.9 ± 0.5
2	Indomethacin	0 ± 0.2	9 ± 1	0.2 ± 0.3
3	scIL-1 and indomethacin	1.7 ± 0.1	14 ± 9	0.2 ± 0.3

[a]Groups of mice were subcutaneously implanted by trocar with indomethacin pellets that release 2.5 μg of drug per day. Indomethacin mice were rested 48 h before i.v. injection of scIL-1 (50 units).
[b]Core body temperature was analyzed 20 min following IL-1 injections. The normal range of core body temperature was 36.6 ± 0.12°C.
[c]Peripheral blood samples were obtained 24 h post injection and plasma samples were analyzed for serum amyloid-P (SAP) levels by radial immunodiffusion. SAP values obtained from normal mice were 60 ± 3 μg/ml.
[d]PMN were quantitated from 24-h postinjection peripheral blood samples. Normal values were 1.58×10^6 ± 0.04 cells/ml.

Table VII. Pretreatment of Animals With Indomethacin Abrogates the scIL-1-Induced Depression of Contact Hypersensitivity Responses

Group #	Treatment[a]	Sensitize[b]	Challenge[b]	Δ Ear Swelling[c]	Percent Depression[d]
1	None	—	+	5 ± 2	—
2	None	+	+	68 ± 2	0
3	Indomethacin	+	+	62 ± 2	8
4	scIL-1	+	+	31 ± 2	59
5	Indomethacin plus scIL-1	+	+	60 ± 2	13

[a]Groups of mice were subcutaneously implanted with indomethacin pellets that release 2.5 μg of drug/day. Indomethacin mice were rested 48 h before i.v. injection of scIL-1 (50 units).

[b]All mice were sensitized to DNFB by applying 25 μl of a 0.25% solution of DNFB in acetone:olive oil (4:1) to their ventral surface. Five days following sensitization these animals were ear-challenged with 10 μl of 0.25% DNFB.

[c]Twenty-four hours following challenge, ear swelling (1×10^{-4} inches ± SEM) was measured with an engineer's micrometer. Results are expressed as the thickness of the challenged ear minus the thickness of the unchallenged ear.

[d]Percent depression was determined using the following equation:

$$\% \text{ depression} = 1 - \frac{\text{swelling (experimental)} - \text{swelling (negative control)}}{\text{swelling (positive control)} - \text{swelling (negative control)}} \times 100.$$

DISCUSSION

The exposure of experimental animals, and presumably humans, to the effects of UVR [6–8], LPS [6], burn trauma [14], or other types of inflammatory stimuli [15–17] is known to result in alterations of their immunologic function. Contact hypersensitivity caused by the topical application of skin-reactive chemicals has been employed as a model experimental system in many of these studies, with reductions in the intensity of the CH response being used as an indicator of depression or suppression of immunologic responsiveness [1,2]. The presence of antigen-specific suppressor T lymphocytes in the spleens of the contact-sensitized, experimental animals has served as the basis for concluding that the dominance of active suppression is responsible for the immunologic changes observed following UVR exposure [18–21].

Most types of inflammatory responses, regardless of the inducing stimulus, are paralled by physiologic changes that are believed to be caused by the action of IL-1 [10,11]. This knowledge led us to question whether some of the immunologic alterations observed in animals undergoing a severe inflammatory response might be mediated through a common IL-1-dependent mechanism. We found that mice given a pyrogenic dose of semi-purified, or recombinant IL-1, exhibited CH responses that were far less intense than those observed in normal animals, supporting the possibility of a common pathway for inflammation-associated depression in immune responsiveness.

Studies undertaken to establish the mechanism(s) responsible for the depression in CH responses determined that both the normal and IL-1-pretreated animals exhibited equivalent levels of CH-effector-cell as well as suppressor-cell activity. This is consistent with our recent findings on the mechanisms responsible for UVR-induced immunosuppression [13]. These studies established that UVR-exposed animals, contact sensitized through normal skin sites, were totally normal for CH-effector- and suppressor-cell generation. Finding that IL-1-treated animals exhibit minimal CH responses following the adoptive transfer of CH-effector cells obtained from normal, antigen-primed donors suggests that the changes caused by IL-1 associate with the effector arm of the CH response. This observation was extended by a demonstration of a reduction in the number of radiolabeled ef-

Table VIII. Depression in Contact Hypersensitivity Responses Caused by scIL-1 or Human Recombinant IL-1_β Is Abrogated by Treating the Adoptive Recipients With Indomethacin

	Treatment of Recipient Mice					
Experiment #	Adoptive Transfer of DNFB-Sensitized Lymphocytes[a]	IL-1[b]	Indomethacin[c]	Challenge[d]	Δ Ear Swelling[e]	Percent Depression[f]
1	—	—	—	+	6 ± 2	—
	+	—	—	+	36 ± 1	0
	+	+	—	+	15 ± 1	70
	+	+	+	+	34 ± 1	7
2	—	—	—	+	3 ± 1	0
	+	—	—	+	24 ± 2	0
	+	+	—	+	10 ± 0	67
	+	+	+	+	29 ± 3	0

[a]DNFB-sensitized lymphocyte populations from animals prepared as adoptive transfer donors as described in *Materials and Methods*. Each recipient animal received 30×10^6 cells.

[b]Recipient mice received 50 units of scIL-1 (Exp. 1) or 150 ng of human recombinant IL-1_β (Exp. 2) 24 h before receiving DNFB-sensitized lymphocytes.

[c]Groups of mice were subcutaneously implanted with indomethacin pellets that release 2.5 μg of drug/day. Indomethacin mice were rested 48 h before i.v. injection of IL-1.

[d]Recipient mice were ear-challenged with 10 μl of a 0.25% solution of DNFB in acetone:olive oil (4:1) immediately following adoptive transfer.

[e]Twenty-four hours following challenge, ear swelling (1×10^{-4} inches ± SEM) was measured with an engineer's micrometer. Results are expressed as the thickness of the challenged ear minus the thickness of the unchallenged ear.

[f]Percent depression was determined using the following equation:

$$\% \text{ depression} = 1 - \frac{\text{swelling (experimental)} - \text{swelling (negative control)}}{\text{swelling (positive control)} - \text{swelling (negative control)}} \times 100.$$

Table IX. Interleukin 1 Appears to Exert its Immunomodulatory Effects on Contact Hypersensitivity Responses by its Capacity to Stimulate Prostaglandin Production

Group	Adoptive Transfer of Sensitized Lymphocytes[a]	Pretreatment of Recipient Mice				Δ Ear Swelling[f]	Percent Depression[g]
		IL-1[b]	AA[c]	PGE_2[d]	Indomethacin[e]		
1	—	—	—	—	—	2 ± 0.4	0
2	+	—	—	—	—	22 ± 0.4	0
3	+	+	—	—	—	8 ± 1.0	70
4	+	+	—	—	+	22 + 1.0	0
5	+	—	+	—	—	8 ± 1.0	70
6	+	—	+	—	+	21 ± 1.0	5
7	+	—	—	+	—	8 ± 1.0	70
8	+	—	—	+	+	9 ± 1.0	65

[a]Normal C3H/HeN strain mice or mice pretreated with IL-1, arachidonic acid (AA), or prostaglandin E_2 (PGE_2) received an i.v. injection of 30 × 10^6 lymph node lymphocytes from DNFB-primed normal syngeneic donors as described in *Materials and Methods*. Recipient mice were ear-challenged with 10 μl of a 0.25% DNFB solution in 4:1 acetone:olive oil.

[b]Recombinant IL-1 (150 ng) was i.v. injected 24 h before the adoptive transfer of sensitized lymphocytes.

[c]Arachidonic acid pellets were subcutaneously implanted 72 h before adoptive transfer of sensitized lymphocytes. Each pellet has the capacity to slowly release 375 μg/day of arachidonic acid over a 20-day period.

[d]Mice were given subcutaneous injections of 10 μg PGE_2 48 and 24 h before adoptive transfer of sensitized lymphocytes.

[e]Indomethacin pellets were implanted 72 h before adoptive transfer of sensitized lymphocytes. Each pellet has the capacity to slowly release 1.25–2.5 μg/day of indomethacin over a 20-day period.

[f]Twenty-four hours following challenge, ear swelling was measured with an engineer's micrometer. Results are expressed as the difference between challenged ear minus unchallenged ear.

[g]Percent depression was calculated using the following equation:

$$\% \text{ depression} = 1 - \frac{\text{swelling (experimental)} - \text{swelling (negative control)}}{\text{swelling (positive control)} - \text{swelling (negative control)}} \times 100.$$

fector cells capable of entering the site of skin challenge following IL-1 pretreatment. We believe that these results indicate that IL-1 is capable of altering the effector arm of the CH response through its capacity to diminish the ability of sensitized effector cells to enter sites of antigen deposition. Clearly, this represents a very simple and logical type of immune regulation and probably involves changes in endothelial cell receptiveness for antigen-activated lymphocytes. It is well recognized that endothelial cells are responsive to the action of IL-1 [22–24] and are also capable of producing this cytokine [23,24].

The treatment of mice with a low, continuously administered dose (1.25–2.5 μg/day) of indomethacin was found to abrogate the capacity of IL-1 to diminish host responses to CH induction. This finding indicates that the stimulated generation of prostaglandins by IL-1 [25,26] is in some way involved in the alteration in CH responses observed. This finding is totally consistent with our recent observations that indomethacin therapy was capable of abrogating UVR-induced depression in CH responses [13].

We have recently concluded a series of experiments that have determined that UVR- and LPS-, as well as IL-1-induced changes in CH responses are all mediated at the efferent level and, furthermore, are all reversible by treatment with the drug indomethacin (R. Daynes et al, manuscript in preparation). Finally, our results that demonstrate that the administration of arachidonic acid or prostaglandin E_2 to normal mice causes a reduction in their capacity to mediate CH responses subsequent to the adoptive transfer of hapten-primed CH-effector cells, adds substantial support to our hypothesis. This finding is similar to the recent observation by Rheins and Nordlund [27], who demonstrated that the topical application of arachidonic acid to normal mice causes a reduction in their capacity to elicit CH responses following skin sensitization to CS agents through the exposed sites.

Herein we describe a prostaglandin-dependent mechanism capable of interfering with the capacity of lymphocytes to localize to peripheral tissue sites. This provides a very general means to control those aspects of cell-mediated immune responses that are dependent on anatomic compartmentalization to achieve effector function. Having established a close linkage with inflammation and IL-1 production, it is quite possible that a similar mechanism may be functioning in other types of experimental and clinical conditions. Immunodepression found in experimental animals with liver damage [16] or burn trauma [14], as well as animals having circulating antigen-antibody complexes [28,29] may all be due to a similar type of alteration in lymphocyte localization potential.

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