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*J Immunol* 1999; 162:6141-6147; 
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IL-1 Is an Effective Adjuvant for Mucosal and Systemic Immune Responses When Coadministered with Protein Immunogens

Herman F. Staats and Francis A. Ennis, Jr.

Mucosal immunization with soluble protein Ag alone may induce Ag-specific tolerance, whereas mucosal immunization with Ag in the presence of a mucosal adjuvant may induce Ag-specific systemic and mucosal humoral and cell-mediated immune responses. The most widely used and studied mucosal adjuvant is cholera toxin (CT). Although the mechanism of adjuvanticity of CT is not completely understood, it is known that CT induces mucosal epithelial cells to produce the proinflammatory cytokines IL-1, IL-6, and IL-8 and up-regulates macrophage production of IL-1 and the costimulatory molecule B7.2. Because IL-1 may duplicate many of the activities of CT, we evaluated IL-1α and IL-1β for their ability to serve as mucosal adjuvants when intranasally administered with soluble protein Ags. IL-1α and IL-1β were as effective as CT for the induction of Ag-specific serum IgG, vaginal IgG and IgA, systemic delayed-type hypersensitivity, and lymphocyte proliferative responses when intranasally administered with soluble protein Ag. Our results indicate that IL-1α and IL-1β may be useful as mucosal vaccine adjuvants. Such an adjuvant may be useful, and possibly required, for vaccine-mediated protection against pathogens that infect via the mucosal surfaces of the host such as HIV. The Journal of Immunology, 1999, 162: 6141–6147.

Mucosal immune responses (secretory IgA, mucosal T helper, and CTL) may play a crucial role in host protection against infectious agents that infect via the mucosal surfaces of the host, including HIV, HSV, respiratory syncytial virus, Bordetella pertussis, and Salmonella typhimurium (1). A major obstacle in the development effective mucosal vaccines is the identification of a safe and effective mucosal adjuvant. Indeed, mucosal (gastric, nasal, rectal, etc.) immunization with soluble protein or peptide immunogens in the absence of mucosal adjuvants may induce a state of Ag-specific immunological tolerance known as oral tolerance or, more appropriately, mucosally induced tolerance (2–8). In contrast, mucosal immunization with soluble protein Ags or peptides coadministered with a mucosal adjuvant such as cholera toxin (CT),3 pertussis toxin (PT), or heat-labile toxin (LT) induces potent systemic and mucosal, humoral, and cell-mediated immune responses (9–15).

The most potent and best-studied mucosal adjuvant is CT (13,16–19). However, CT is not safe for use as a mucosal adjuvant in humans because as little as 5 μg of CT causes massive diarrhea when intragastrically administered to human volunteers (20). Although the use of CT as a mucosal adjuvant in research animals is generally well tolerated, in some cases the use of CT was associated with the production of Ag-specific IgE responses and lethal anaphylactic reactions (21,22). To repress the toxicity associated with toxin adjuvants, mutant CT, LT, and PT molecules have been produced that exhibit reduced or undetectable toxic activity while maintaining mucosal adjuvant activity (15,23–28). Although these molecules possess potent adjuvant activity in the absence of toxicity, they maintain immunogenic properties when administered to experimental animals (23,29,30). Unfortunately, the immunogenicity of these mutant toxin molecules may also prevent their widespread repeated use as mucosal adjuvants because pre-existing immunity to CT reduces its adjuvant activity (31,32).

Exactly how CT (or LT or PT) acts as a mucosal adjuvant is not completely understood. When used as a mucosal adjuvant, CT has been reported to facilitate Ag access to the mucosal immune system (31), enhance the induction of Ag-specific T and B cells (33,34), and up-regulate the expression of the costimulatory molecule B7.2 on mucosal APC (35). The adjuvant activity of CT has also been associated with its ability to induce cytokine secretion by a variety of cell types (Table I). For example, treatment of the P388D1 M* cell line with CT enhanced the production of IL-1 and the APC activity of the cells in an allogeneic T cell proliferation assay (36). Treatment of CT-stimulated P388D1 cells with anti-IL-1α completely abrogated the CT-enhanced APC activity (36). CT also enhanced IgA production in surface IgA– spleen B cells, and CT-enhanced IgA production could be blocked by anti-TGF-β Abs (37). Therefore, CT-enhanced APC activity in macrophages and IgA production by B cells can be attributed to CT-stimulated production of IL-1α and TGF-β, respectively (36,37). Additionally, the CT-induced cytokines IL-1 and TGF-β may duplicate many responses induced by CT (37–44) (Table I). A safe and effective mucosal adjuvant is clearly needed to aid the development of effective mucosal vaccines. Although CT is an effective mucosal adjuvant, its toxicity and immunogenicity may preclude its use in humans (see above). Because many of the activities induced by CT could be duplicated with specific cytokines (Table I), our hypothesis is that the adjuvanticity of CT is due to its ability to induce the local production of proinflammatory cytokines that create an immunological microenvironment suitable for the induction of Ag-specific immune responses.

Received for publication November 2, 1998. Accepted for publication February 26, 1999.

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1Abbreviations used in this paper: CT, cholera toxin; PT, pertussis toxin; LT, heat-labile toxin; TT, tetanus toxoid; DTH, delayed-type hypersensitivity.

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0022-1767/99/$02.00
for the induction of systemic and mucosal immune responses. Because IL-1 secretion was induced by CT, IL-1 was responsible for CT-enhanced APC function of macrophages, IL-1 duplicates many of the activities of CT, and IL-1 is a potent proinflammatory cytokine that induces the production of many additional cytokines, we formed the additional hypothesis that coadministration of IL-1 and IL-1, exhibited mucosal adjuvant activity when intranasally administered with soluble protein Ags. Our results indicated that IL-1 was as effective as cholera toxin for the induction of Ag-specific systemic and mucosal immune responses.

**Materials and Methods**

**Animals**

Female BALB/c and C57BL/6 mice, 16–18 g, were purchased from Frederick Cancer Research and Developmental Center, National Cancer Institute (Frederick, MD). Animals were housed in filter-top cages and provided food and water ad libitum. All procedures for use and care of mice were approved by Duke University’s institutional animal care and use committee.

**Immunization**

Mice were intranasally immunized as previously described (10, 11, 14). Briefly, mice (three or four mice per group) were intranasally immunized with the indicated concentration (Table II) of OVA (A-5503, Sigma, St. Louis, MO) or tetanus toxoid (TT; provided by Drs. Dennis Kattok and John Eldridge, Wyeth-Lederle Vaccines and Pediatrics, Pearl River, NY) and the indicated adjuvant in a total volume of 15 µl (7.5 µl/nostril). The mucosal adjuvant CT was obtained from List Biological Laboratories (Campbell, CA). Recombinant human IL-1α and IL-1β were obtained from the National Cancer Institute Biological Resources Branch, Division of Cancer Treatment, Frederick Cancer Research and Development Center. The recombinant human IL-1α had a sp. act. of 3.0 x 10^5 U/mg, while recombinant human IL-1β had a sp. act. of 1.81 x 10^7 U/mg. Ag preparations were diluted to the appropriate concentration in sterile distilled water, and 7.5 µl of the Ag mixture was introduced into each nostril while mice were under isoflurane anesthesia (IsoFlo, USP, Sollav Animal Health, Mendota Heights, MN).

**Sample collection**

Blood was collected from the retro-orbital plexus using a heparinized Natelson capillary tube (Baxter Healthcare, McGaw Park, IL) while mice were under ketamine/xylazine anesthesia. Vaginal wash samples were collected by washing the vaginal cavity with 100 µl of sterile PBS while mice were anesthetized with ketamine/xylazine. This procedure resulted in an

**Table I. Literature reports of the ability of mucosal adjuvants and cytokines to induce cytokine production and enhance APC activity**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Stimulus</th>
<th>IL-1</th>
<th>IL-6</th>
<th>IL-8</th>
<th>IgA Production</th>
<th>APC Activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal epithelial cell lines</td>
<td>CT</td>
<td>↑ ↑</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td>39–44, 54, 75</td>
</tr>
<tr>
<td></td>
<td>CT-B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-1β</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGF-β1</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGF-β1 + IL-1β</td>
<td></td>
<td></td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF-α + IL-1β + TGF-β1</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td></td>
<td>CT + IL-1β</td>
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<td></td>
<td>CT + TNF-α</td>
<td></td>
<td></td>
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<td>M[phi] cell line (P388D1)</td>
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<td>↑</td>
<td>↑</td>
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<td>36</td>
</tr>
<tr>
<td></td>
<td>CT + anti-IL-1α</td>
<td>←</td>
<td></td>
<td>↑</td>
<td>←</td>
<td>←</td>
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<tr>
<td>PBMC</td>
<td>CT</td>
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<td>↑</td>
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<td>←</td>
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<td>CT + anti-TGF-β</td>
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<td>↑</td>
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<tr>
<td></td>
<td>TGF-β1</td>
<td></td>
<td></td>
<td>↑</td>
<td>←</td>
<td>←</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TGF-β1 + anti-TGF-β</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>←</td>
<td>←</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell Type</th>
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<th>IgA Production</th>
<th>APC Activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
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<td>Intestinal epithelial cell lines</td>
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<td></td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>IFN-γ</td>
<td>↑</td>
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<td>←</td>
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<td></td>
<td>TGF-β1</td>
<td></td>
<td></td>
<td>↑</td>
<td>←</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Literature review on the effects of mucosal adjuvants and cytokines on cytokine production and APC activity by intestinal epithelial and macrophage cell lines, PBMC, sIgA B cells, and freshly isolated intestinal epithelial cells. APC activity was determined in alloantigen presentation systems. Symbol legend: ←, no change over unstimulated control cells; ↑, 2- to 10-fold enhancement over unstimulated controls; ↑↑, 10- to 100-fold enhancement over unstimulated controls; ↑↑↑, >100-fold enhancement over unstimulated controls.

**Table II. Immunization protocols to evaluate the mucosal adjuvant activity of IL-1α and IL-1β**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Adjuvant</th>
<th>Adjuvant Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1 (BALB/c)</td>
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<td>None</td>
</tr>
<tr>
<td></td>
<td>CT × 3</td>
<td>CT (1 µg)</td>
</tr>
<tr>
<td></td>
<td>CT × 1</td>
<td>CT (1 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1α × 3</td>
<td>IL-1α (4 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1α × 1</td>
<td>IL-1α (4 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1β × 3</td>
<td>IL-1β (4 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1β × 1</td>
<td>IL-1β (4 µg)</td>
</tr>
<tr>
<td>Expt. 2 (C57BL/6)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>CT × 3</td>
<td>CT (1 µg)</td>
</tr>
<tr>
<td></td>
<td>CT × 1</td>
<td>CT (1 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1α × 3</td>
<td>IL-1α (4 µg)</td>
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<tr>
<td></td>
<td>IL-1α × 1</td>
<td>IL-1α (4 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1β × 3</td>
<td>IL-1β (4 µg)</td>
</tr>
<tr>
<td></td>
<td>IL-1β × 1</td>
<td>IL-1β (4 µg)</td>
</tr>
</tbody>
</table>

* Immunization protocols to evaluate the mucosal adjuvant activity of IL-1α and IL-1β. There were three to four mice per group, per experiment. For expt. 1, mice were immunized with 100 µg of OVA on days 0, 14, and 14 alone or in the presence of adjuvants as indicated. For expts. 2 and 3, mice were immunized with 50 µg of TT on days 0, 7, and 14 alone or in the presence of adjuvants as indicated. For technical details concerning the immunization, see Materials and Methods.
ELISA
An ELISA was used to determine the presence of Ag-specific Abs in serum and vaginal wash samples. The ELISA was performed as previously reported (10, 11), except that ELISA plates were coated with OVA or TT at 5 μg/ml. The end-point titer represents the reciprocal log₂ dilution of the last sample whose OD was at least 2-fold higher than the OD of a corresponding naive sample. The geometric mean titer and SD were calculated using the log₂ end-point titers and then were transformed to the antilog for figures and tables. All statistical evaluations were performed with the log₂ data. For the small vaginal lavage sample sizes, vaginal IgG and IgA responses are reported as end-point ELISA titers instead of standardizing them based on total IgG and IgA concentrations (10, 11). Detection reagents for the ELISA were purchased from Southern Biotechnology Associates (Birmingham, AL). IgG subclass detection reagents were specific for the indicated IgG subclass (i.e., there was no cross-reactivity among the IgG subclass reagents).

Cell isolation and proliferation assay
Spleens were aseptically removed, and single-cell suspensions were produced by cutting the spleen into small pieces (∼5 mm × 5 mm) and expressing the cells from the splenic capsule with the sterile end of a 5-cc syringe plunger. RBC were removed by centrifugation over Cappell Lymphocyte Separation Medium (ICN, Aurora, OH). Cells were adjusted to 2 × 10^6 cells/ml in complete T cell medium ( RPMI 1640, 10% FBS, HEPES, Pen/Strep, and 2-ME). To an equal volume of cells was added an equal volume of complete T cell medium or medium containing 0.2 μg/ml of the appropriate Ag (OVA or TT) to give a final cell density of 1 × 10⁶ cells/ml and Ag at 0.1 μg/ml. Cells (100 μl) were added to round-bottom 96-well microtiter plates in triplicate and were incubated in a 37°C, 10% CO₂ in air, humidified environment for 5 days. Four to six hours before harvesting, 0.5 μCi of [3H]thymidine (New England Nuclear Research Products, Boston, MA) was added to each well. Cells were harvested onto glass filters using a PHD sample harvester (Cambridge Technology Products, Boston, MA) was added to each well. Cells were harvested onto glass filters using a PHD sample harvester (Cambridge Technology, Watertown, MA). Incorporation was determined by placing the filters in ScintiVerse BD scintillation fluid (Fisher, Pittsburgh, PA) and counting with a 2000CA Tri-Carb liquid scintillation analyzer (Packard, Downers Grove, IL).

Delayed-type hypersensitivity (DTH)
For the measurement of DTH responses, an ear swelling assay was employed as previously described (10, 11). Briefly, 25 μg of Ag was injected into the right ear in 10 μl of sterile PBS while 10 μl of sterile PBS was injected into the left ear as a control. Ear swelling was measured 24–48 h after injection with a dial thickness gauge (Mitutoyo thickness gauge #7326, Kawasaki, Japan). Ag-specific ear swelling was calculated by subtracting the ear swelling of the PBS-injected ear from the swelling of the Ag-injected ear. DTH ear-swelling units are 10⁻² inches (i.e., 1 DTH unit = 1 × 10⁻² inches).

Statistical analysis
Statistical significance was determined using ANOVA, with comparison of multiple means using Bonferroni’s (Dunn) t tests, Sidak’s t tests, and Tukey’s Studentized range test on SAS software (SAS Institute, Cary, NC). A difference was considered significantly different if two of three tests indicated a significant difference. The level of significance used was 0.05. Error bars represent the SD.

Results
IL-1α and IL-1β enhance serum Ag-specific IgG responses when intranasally administered with soluble protein Ags
To determine whether IL-1α or IL-1β possessed mucosal adjuvant activity, BALB/c or C57BL/6 mice were intranasally immunized with Ag alone or with Ag in the presence of CT, IL-1α, or IL-1β (Table II). In Expt. 1, BALB/c mice were intranasally immunized with 100 μg of OVA on days 0, 14, and 28 in the absence of adjuvants or in the presence of 1 μg of CT, 4 μg of IL-1α, or 4 μg of IL-1β administered with all immunizations or with the first immunization only (Table II). Both IL-1α and IL-1β were able to serve as mucosal adjuvants when intranasally administered with OVA with all immunizations (three times) or with the first immunization only (p < 0.05 compared with no adjuvant control).

When coadministered with all three immunizations, the day 42 serum anti-OVA IgG titers for the IL-1α, IL-1β, and CT groups were 1/262,144, 1/104,031, and 1/13,004, respectively (Fig. 1, Expt. 1). IL-1α and IL-1β were also effective adjuvants when administered with the first immunization only, resulting in serum anti-OVA IgG titers of 1/26,008 and 1/13,004, respectively. Anti-OVA IgG titers were 1/100 in the sera collected from mice immunized with no adjuvant or with CT administered with the first immunization only (Fig. 1, Expt. 1). IL-1α and IL-1β were also tested at a 1.3-μg dose (3-fold dilution of 4 μg) administered with all three nasal immunizations (days 0, 14, and 28). IL-1α at 1.3 μg induced a day 42 serum anti-OVA IgG end-point titer of 1/46,341, while IL-1β at 1.3 μg induced a day 42 serum anti-OVA IgG end-point titer of 1/2,048 (data not shown). Because IL-1α and IL-1β were more effective at the 4-μg dose, subsequent studies used only the 4-μg dose.

To determine whether IL-1α and/or IL-1β exhibited mucosal adjuvant activity when coadministered with a more relevant vaccine Ag, C57BL/6 (Expt. 2) or BALB/c (Expt. 3) mice were intranasally immunized with 50 μg of TT alone or in the presence of adjuvants (Table II). To comply with other published mucosal immunization protocols (9, 22), mice were immunized on days 0, 7, and 14 for Expt. 2 and 3 (Table II). IL-1α and IL-1β were effective mucosal adjuvants when intranasally administered with 50 μg of TT on days 0, 7, and 14 in both C57BL/6 (Expt. 2) and BALB/c (Expt. 3) mice (p < 0.05 compared with no adjuvant control).
FIGURE 2. Vaginal Ag-specific IgA end-point titers (reciprocal) after intranasal immunization as indicated in Table II. There were three or four mice per group in each experiment. #1, Expt. 1; #2, Expt. 2; #3, Expt. 3. For Expt. 1, BALB/c mice were immunized on days 0, 14, and 28 with 100 μg OVA. For Expt. 2 (C57BL/6) and Expt. 3 (BALB/c), mice were immunized on days 0, 7, and 14 with 50 μg TT. Sera from three to four mice per group, per experiment, were pooled and assayed for Ag-specific IgG1, IgG2a, IgG2b, or IgG3 end-point titers.

When administered with all three immunizations, day 21 serum anti-TT titers for IL-1α and IL-1β groups were 1/155,872 and 1/208,064 for C57BL/6 mice, respectively, and 1/165,140 and 1/208,064, respectively, for BALB/c mice (Fig. 1, Expt. 2 and 3). IL-1α also exhibited significant adjuvant activity when intranasally administered to BALB/c mice with TT on day 0 only (p < 0.05 compared with no adjuvant control). Within the same experiment, there were no significant differences between any of the CT, IL-1α, or IL-1β adjuvant groups.

Ag-specific IgG subclass end-point titers were measured to compare the Ag-specific IgG subclass responses induced by IL-1α and IL-1β to those induced by CT. The Ag-specific IgG subclass profiles associated with the use of IL-1α or IL-1β as a mucosal adjuvant were comparable to those induced by the use of CT as a mucosal adjuvant (Table III).

**IL-1α and IL-1β induce mucosal IgA responses when used as a mucosal adjuvant**

Vaginal lavage samples were monitored for the presence of Ag-specific IgG and IgA to determine whether IL-1α or IL-1β could induce Ag-specific IgA responses when used as a mucosal vaccine adjuvant. In all experiments IL-1α and IL-1β were as effective as CT in their ability to induce the production of Ag-specific mucosal IgA and IgG responses (Fig. 2). In Expt. 1 when the adjuvants were used with all three immunizations, the day 42 vaginal anti-OVA IgA titers for the IL-1α and IL-1β groups were 1/32 and 1/128, respectively, compared with an undetectable anti-OVA IgA response in the CT group (Fig. 2, Expt. 1). Even when used with the first immunization only, IL-1α and IL-1β induced anti-OVA IgA responses that were detectable in vaginal lavage samples (Fig. 2, Expt. 1). In Expt. 2 and 3, the use of IL-1α and IL-1β with all immunizations consistently induced Ag-specific vaginal IgA titers that were 2- to 8-fold higher than those induced by CT (Fig. 2, Expt. 2 and 3). When administered with TT for the first immunization only, IL-1α (Expt. 3) and IL-1β (Expt. 2 and 3) induced the production of anti-TT vaginal IgA responses.

**IL-1α and IL-1β induce cell-mediated immune responses when used as a mucosal adjuvant**

Ear-swelling DTH responses were used as an indicator of in vivo cell-mediated immune responses after intranasal immunization with soluble protein Ags alone or in the presence of CT, IL-1α, or IL-1β. When intranasally administered with OVA for all three immunizations (Expt. 1), IL-1α and IL-1β induced DTH ear-swelling responses (114 ± 8.9 and 106 ± 7.6 DTH units, respectively) that were significantly greater than those induced by any other group (p < 0.05; Fig. 3). In subsequent studies, IL-1α (Expt. 3) and IL-1β (Expt. 2) induced DTH ear-swelling responses that were significantly greater than those induced by immunization in the absence of adjuvant (p < 0.05) and were comparable to those induced by CT. The use of IL-1 with the first immunization only did not induce significantly elevated DTH ear-swelling responses.
DTH ear-swelling units are 10^-2 with soluble protein Ags. IL-1α able to serve as mucosal adjuvants when intranasally administered.

Results from the present study indicate that IL-1α and IL-1β induced lymphocyte proliferative responses comparable to those induced by CT (Fig. 4).

Discussion

Results from the present study indicate that IL-1α and IL-1β are able to serve as mucosal adjuvants when intranasally administered with soluble protein Ags. IL-1α and IL-1β were as effective as CT for the induction of Ag-specific serum IgG, vaginal IgA, as well as in vivo and in vitro cell-mediated immune responses. Although numerous reports have indicated that IL-1 is able to serve as an adjuvant for the induction of systemic Ag-specific immune responses (45–51), this is the first report demonstrating mucosal adjuvant activity for IL-1α when intranasally administered with soluble protein Ags.

A number of observations support the hypothesis that the production and release of IL-1 by CT-stimulated mucosal epithelial cells are critically involved in the mucosal adjuvant activity of CT. CT and the related LT are heterohexameric proteins with a m.w. of approximately 85,000 (52). The toxin is composed of one CT-A subunit noncovalently associated with five CT-B subunits. CT-B is responsible for binding to its receptor, ganglioside GM1, while the CT-A subunit possesses the toxic ADP-ribosyltransferase activity (52). Our hypothesis for CT-induced, IL-1-mediated mucosal adjuvant activity is as follows. After mucosal administration as an adjuvant, CT binds to its specific receptor on the surface of mucosal epithelial cells. Indeed, orally administered LT-B (similar to CT-B) bound to the brush border of villous enterocytes as well as epithelial cells overlying the Peyer’s patches (53). After binding to GM1, CT induces the production and/or secretion of IL-1 from mucosal epithelial cells (54). CT-induced IL-1 binds to type I IL-1R on mucosal epithelial cells (55, 56) and stimulates the production of more IL-1 as well as other cytokines (IL-6, IL-8, TGF-β) that affect both mucosal epithelial cells and other cell types (fibroblasts, macrophages, dendritic cells) and enhance the induction of mucosal and systemic immune responses. IL-1 stimulation down-regulates type I IL-1R expression on mucosal epithelial cells (57) and therefore limits the proinflammatory action of IL-1 induced by mucosal administration of CT. Indeed, mucosal adjuvant activity was observed when CT was coadministered with specific Ag, but not when it was administered 24 h before or after Ag dosing (58).

IL-1α and IL-1β are proinflammatory cytokines produced by many cell types including macrophages, PBMC, fibroblasts, and intestinal epithelial cell lines (36, 54, 59–62). IL-1α and IL-1β use the same cell surface receptors, type I and type II, although only the type I IL-1R transduces a signal in response to ligation of the receptor with IL-1 (59, 63). IL-1-induced IL-1 production of itself as well as a large number of other proteins, including IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-12, TGF-β, TNF-α, TNF-β, GM-CSF, G-CSF, M-CSF, and the IL-2R (39, 61). Because of its ability to induce the production of cytokines involved in the induction of immune responses, the potential of IL-1α as a vaccine adjuvant is apparent.

IL-1 has been extensively studied in humans and has been found to be extremely toxic after i.v. administration (61, 65–70). With both IL-1α and IL-1β, the predominant side effects observed were fever, chills, hypotension, and nausea. In these human studies, IL-1 was administered by the i.v. route and was administered daily for...
4–14 days. In published murine studies, systemic administration of IL-1α and IL-1β also exhibited adverse side effects, such as fever and weight loss (71–73). The use of other cytokines as systemically administered adjuvants has also been reported to have adverse side effects in murine models. For example, i.p. injection of IL-12 and IL-18 was lethal when tested as an anti-tumor therapy at the doses of 0.01 and 1.0 μg, respectively, although a protective, nonlethal effect was observed at doses of 0.01 and 0.2 μg, respectively (74). In the present murine study, noticeable side effects were not observed after nasal administration of IL-1α or IL-1β (i.e., no cytokine-induced mortality, no obvious weight loss or abnormal behavior). Additionally, IL-1α and IL-1β were administered locally and were effective adjuvants when administered at weekly (Expt. 2 and 3) or biweekly intervals (Expt. 1). In some cases, IL-1 was an effective adjuvant when administered with only the first immunization in a series of immunizations. Because IL-1 stimulation of mucosal epithelial cells down-regulates expression of the type II IL-1R, the inflammatory activity of IL-1 used as a mucosal adjuvant may be minimized (57). Additional studies to carefully monitor the effects of nasally administered IL-1α and IL-1β on body temperature, body weight, and histology of the upper respiratory tract are currently being performed in our laboratory.

IL-1α and IL-1β may be superior to CT and related molecules as mucosal adjuvants because they are self proteins. Although CT is a potent mucosal adjuvant, the fact that it is very immunogenic in addition to being toxic may diminish its effectiveness when repeatedly used as a mucosal adjuvant. Indeed, pre-existing immunity to CT reduces its effectiveness as a mucosal adjuvant (31, 32). Despite having reduced toxicity in the presence of adjuvant activity, mutant CT molecules maintain their immunogenicity, which may reduce their effectiveness when repeatedly used as mucosal adjuvants in humans (23, 29, 30). In contrast to CT, IL-1α and IL-1β are self proteins and therefore nonimmunogenic. This quality may allow IL-1α or IL-1β to be repeatedly used as an effective mucosal adjuvant. Because human IL-1α and IL-1β were used in the present study and are expected to be immunogenic in mice, it is impossible to determine what effect, if any, host immunity to the mucosal adjuvants (CT, IL-1α, IL-1β) had on the induction of systemic and mucosal immune responses in this study. Additional studies are being performed with CT and recombinant murine IL-1α and IL-1β (expected to be nonimmunogenic in mice) to determine whether the repeated use of host proteins (i.e., IL-1) is superior to the use of foreign proteins as mucosal adjuvants.

The development of safe and effective mucosal adjuvants is critical to the development of mucosal vaccines for use in humans. Of particular interest to our laboratory is the development of vaccines that induce mucosal anti-HIV humoral and cell-mediated immune responses (10, 11, 14). The results from the present study suggest that 1) IL-1α and IL-1β are effective mucosal adjuvants for the induction of systemic and mucosal immune responses and 2) cytokines are biologically active when intranasally administered. The use of recombinant cytokines as mucosal adjuvants may prove useful for the induction of systemic and mucosal anti-HIV immune responses in humans. Studies are being performed to evaluate the use of IL-1α and IL-1β as vaccine adjuvants when intranasally administered with HIV peptide and protein immunogens.

Acknowledgments

We thank Neil Sparks for expert laboratory assistance and critical review of the manuscript; Drs. Francis Ennis, Jerry R. McGhee, Alice Pilgrim, and Curtis Bradley for critical review of the manuscript; and Wyeth-Lederle Vaccines and Pediatrics for their kind donation of TT.


