Activation of Macrophages in an Experimental Rat Model of Arthritis Induced by *Erysipelothrix rhusiopathiae* Infection

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Infection of Lewis rats with *Erysipelothrix rhusiopathiae* represents an experimental model system of acute and chronic arthritis. We studied here the acute inflammatory phase with respect to stimulation of macrophages and lymphocytes. Intragluteal injection of viable *E. rhusiopathiae* (10^7 to 10^8 bacteria) rapidly induced generalized inflammation, loss of body weight, hind leg arthritis, and systemic macrophage activation within 2 to 3 days. The same symptoms could also be evoked by injection of dead *E. rhusiopathiae*. Ex vivo, peritoneal macrophages released large amounts of tumor necrosis factor alpha on day 2 and interleukin-1 on day 3, whereas production of prostaglandin E2 was delayed to days 5 to 7 and appeared to counteract tumor necrosis factor alpha synthesis. The inflammatory response and development of arthritis were strongly dependent on T lymphocytes, as evidenced by the following findings: (i) lymphocytes released lymphokines that activated macrophages to enhanced mediator release; (ii) treatment of rats with cyclosporin A reduced infection-induced macrophage activation; (iii) mitogen-stimulated thymocyte proliferation was enhanced, indicating an infection-induced maturation-differentiation process in the thymus; and (iv) in T-cell-deficient nude rats, a higher dose of bacteria was required for infection, the inflammatory response was less severe, and only mild, but not chronic, arthritis developed. Thus, an *E. rhusiopathiae*-induced inflammation in rats provides a useful tool to characterize activated macrophages and T lymphocytes during the development of acute arthritis and its transition into the chronic form.

Rheumatoid arthritis is a disease of unknown etiology, which locally affects joints and systemically alters leukocyte functions (17, 18, 21, 23, 25, 31, 36, 37, 47, 52). Several reports have indicated that a systemic activation of leukocytes, particularly of macrophages, may be responsible for rheumatoid arthritis symptoms, as shown, for example, by enhanced prostaglandin E2 (PGE2) and interleukin-1 (IL-1) release (7, 8, 11, 17, 22, 29, 34, 36, 37, 40). The underlying mechanism for the persistent macrophage activation is unknown but may be related to a continuous release of cytokines from lymphocytes or macrophages stimulated by an unknown agent.

Most experimental research has utilized adjuvant- or collagen-induced arthritis to compare patterns of tissue destruction and leukocyte reactivity with human rheumatoid arthritis (19, 41, 42, 45, 46). Arthritis may also develop after infection with bacteria (1, 5, 20, 24, 38). Among arthritis-inducing bacteria, gram-positive *Erysipelothrix rhusiopathiae* is a natural pathogen of swine and initially induces an acute infection that rapidly generalizes into a septicemic phase with high fever and weight loss, which is then followed by a self-sustaining, chronic arthritis (43, 49, 50). Since *E. rhusiopathiae* produces a similar disease in rats (51), it represents a convenient model to study the sequence of events that lead from infection to arthritis. In this report we will characterize macrophage activation during the acute inflammatory response with respect to release of tumor necrosis factor alpha (TNF-α), IL-1, and PGE2, all of which have previously been shown to mediate acute inflammatory reactions (3, 9, 12, 33). Subsequent reports will deal with infection-free chronic arthritis and transfer of the disease by lymphocytes.

**MATERIALS AND METHODS**

**Rats.** Lewis and T-cell-deficient Han-rnu rats 6 to 8 weeks of age were obtained from the Zentrale Versuchstieranstalt, Hannover, Federal Republic of Germany. All rats were free from pathogenic virus infections. Susceptibility to infection, development of inflammation, and arthritis were equal in male and female rats.

**Bacteria.** *E. rhusiopathiae* T 28 was provided by W. Leibold, Veterinary Medical School, Hannover, Federal Republic of Germany. Bacteria were grown on blood agar in bacteriological petri dishes. After harvest, *E. rhusiopathiae* bacteria were washed several times in physiologic saline. After samples were removed for colony counts, bacteria were stored without loss of viability at 4°C for injection on the next day. Colony counts were performed on the next day and the stored *E. rhusiopathiae* suspension was adjusted to the required bacterial concentration. Kiling of *E. rhusiopathiae* was performed by two sonifications for 1.5 min at 300 W. No bacteria survived, as tested by plating on blood agar. For selected experiments *Escherichia coli* B (NCTC 10537) was employed.

**Infection of rats.** Except for one set of experiments, Lewis rats were employed for all studies. By using ether anesthesia, rats were intramuscularly injected in the right gluteal area with 10^8 *E. rhusiopathiae* bacteria suspended in 1 ml of physiologic saline. Control rats received an injection of 1 ml of physiologic saline only. In selected experiments rats were treated with indomethacin suspended in drinking water and the dose was adjusted to 10 mg/kg per day. Treated rats were monitored regularly with respect to food and water intake, body weight, symptoms of inflammation (ruffled fur, hunched back, and lethargy), and development of arthritis in fore and hind legs.

**75Se labeling.** *E. rhusiopathiae* (5 x 10^7 bacteria per ml) was precultured for 8 h in RPMI 1640 medium free from

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antibiotics and methionine but supplemented with 10% fetal calf serum. Thereafter, 3 µCi of 35Se-methionine (specific activity, 4 Ci/mmol; Amersham-Buchler, Braunschweig, Federal Republic of Germany) per ml was added for 8 h. At the end of incubation, bacteria were washed four times and incubated for an additional 30 min in physiologic saline to permit release of free radioactivity that was not incorporated into bacterial products. Samples of 105 35Se-labeled E. rhusiopathiae bacteria were suspended in 1 ml of physiologic saline and intramuscularly injected into the right gluteal region of Lewis rats. On days 4 and 11 after injection, blood was collected, various organs were removed, exsanguinated by perfusion with saline, and freed from adjoining tissue, and radioactivity of whole organs or blood samples was determined in a gamma counter. One sample of noninjected 107 radiolabeled E. rhusiopathiae bacteria was separately counted at each time point of the experiment. During the observation period of 11 days, activity of the radiolabel declined from 100 to 95.7%.

Treatment with CsA. Cyclosporin A (CsA; kindly provided by J. F. Borel, Sandoz, Basel, Switzerland) was dissolved in pure olive oil at a final concentration of 2 mg/ml. Infected and noninfected rats received daily intramuscular injections at a concentration of 5 mg/kg of body weight, and control rats were injected with the solvent only.

Cells and culture conditions. Peritoneal macrophages were harvested by washing the peritoneal cavity with cold, pyrogen-free physiologic saline. Cells were washed twice in saline and suspended in serum-free RPMI 1640 medium supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), and 2-mercaptoethanol (10 mM). Macrophages were seeded into 24-well culture plates (Nunc, Wiesbaden, Federal Republic of Germany) and allowed to adhere for 1 h at 37°C in a 5% CO2-95% air atmosphere. Thereafter, the monolayers were washed three times to remove the nonadherent cells. The remaining cells consisted of >95% macrophages, as determined by phagocytosis of carbon particles and staining for nonspecific esterase (15, 30). Incubation of macrophages in RPMI 1640 medium (0.5 x 106/ml) was carried out for 20 h. Thereafter, the culture supernatants were harvested, rendered cell-free by centrifugation, and stored at −20°C until assay.

Mesenteric lymph nodes and the spleen from noninfected and infected rats were aseptically removed, and single-cell suspensions were prepared by forcing organ fragments through 60-mesh stainless steel sieves. Single-cell suspensions were washed twice and suspended in RPMI 1640 culture medium. Macrophages were removed by 2-h adherence to plastic culture dishes, and thereafter 106 lymphocytes per ml were seeded in 24-well plates for 20 h. At the end of incubation, the culture supernatants were harvested and stored at −20°C until assay.

Determination of TNF-α. The amount of TNF-α in macrophage and lymphocyte culture supernatants was determined by cytotoxicity against TNF-α-sensitive L929 cells (35). Briefly, L929 cells (6 x 104/0.2 ml of RPMI 1640 medium) were seeded in 96-well microtiter plates and grown overnight to establish a dense monolayer. Together with actinomycin D (1 µg/ml) in order to enhance L929 sensitivity to TNF-α, 0.1 ml of diluted or undiluted supernatants of macrophage or lymphocyte cultures was added. After 18 h of incubation, the viability of L929 cells was measured by staining for 1 h with a 20-µl0.2 ml volume of 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/ml). Upon completion of the MTT reaction, culture supernatants were removed and cells were lysed by the addition of 0.1 ml of isopropanol containing 40 mM HCl. The photometric measurement was performed at 540 nm in an MR 600 microplate-autoreader (Dynatech, Denkendorf, Federal Republic of Germany). The TNF-α content in a sample, expressed in picograms per milliliter, was calculated by comparison with a calibration curve established with either murine recombinant TNF-α (kindly provided by G. R. Adolf, Ernst-Boehringer-Institute, Vienna, Austria) or human recombinant TNF-α (kindly provided by BASF/Knoll AG, Ludwigshafen, Federal Republic of Germany). The specificity of TNF-α determinations in macrophage and lymphocyte culture supernatants was corroborated by two means: (i) parallel use of L929 cells rendered TNF-α insensitive by prolonged cultivation in TNF-α-containing medium and (ii) neutralization of TNF-α by a 15-min preincubation with a 1:1,000 dilution of a specific rabbit anti-murine TNF-α antiserum (kindly provided by D. Männe, German Cancer Research Center, Heidelberg, Federal Republic of Germany).

Determination of IL-1. The content of IL-1 in macrophage and lymphocyte culture supernatants was determined by the fibroblast growth-promoting assay, as recently described (16, 28). Briefly, IL-1-sensitive fibroblasts from human foreskin were kindly provided by H. Loppnow, Research Institute, Borstel, Federal Republic of Germany) were precultured in 25-cm2 culture flasks in RPMI 1640 medium. After several days of growth, fibroblasts were detached by EDTA-trypsin, washed, and adjusted to 5 x 104/ml in medium. A 0.1-ml volume of this cell suspension was seeded into flat-bottomed microtiter plates, and after 24 h of preincubation, the medium was replaced by 0.2 ml of diluted or undiluted test samples or appropriately diluted rat IL-1 standard samples (30). After 72 h of incubation, the cells were pulsed for 18 h with 20 µCi of [3H]thymidine (specific activity, 5 Ci/mmol; Amersham-Buchler, Braunschweig, Federal Republic of Germany). For harvesting of the cells, the medium was replaced with 0.1 ml of an EDTA-trypsin solution, and the detached cells were harvested after 10 min by an automated cell harvester. Incorporated radioactivity was measured by liquid scintillation and converted into IL-1 activity (units per milliliter) according to calibration samples. This test system detects IL-1 with higher sensitivity and reliability than the previously used thymocyte costimulation assay (30), and it is insensitive to TNF-α, IL-2, lipopolysaccharide, and prostaglandins.

Determination of PGE2. The amount of PGE2 in culture supernatants was determined by radioimmunoassay, using a highly specific antisera and a double-antibody method as previously described in detail (13).

Lymphocyte proliferation assay. Mitogen-induced lymphocyte proliferation was determined by previously described methods (14, 27). Briefly, single-cell preparations of spleen and thymus from healthy and E. rhusiopathiae-infected rats were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum. Cells (105/0.2 ml) were cultured in round-bottomed microtiter plates with an optimal concanavalin A concentration (3 µg/ml) in the presence or absence of indomethacin (1 µg/ml). After 48 h of incubation at 37°C in a humidified 5% CO2-95% air atmosphere, proliferation of lymphocytes was determined by pulsing the cells for 12 h with 20 µCi of [3H]thymidine (specific activity, 5 Ci/mmol). At the end of incubation, cells were collected onto glass fiber filters by an automated cell harvester, and the incorporation of [3H]thymidine into DNA was measured in a beta scintillation counter.
RESULTS

Clinical signs and symptoms. The primary objective of this study was to characterize the initial inflammatory stage in this animal model of rheumatoid arthritis. When more than 10⁴ E. rhusiopathiae bacteria per rat were injected, usually a fulminant septicemia occurred that led to death within 10 to 12 days. However, with the routinely used E. rhusiopathiae dose of 10⁴ bacteria, the rats survived the acute stage of inflammation and developed arthritis that generally affected the hind leg and infrequently the fore leg joints. E. rhusiopathiae dose-response studies established that concentrations of as low as 10¹ E. rhusiopathiae bacteria per animal were capable of inducing an acute and chronic joint inflammation (data not shown).

After a single intramuscular injection of 10⁴ E. rhusiopathiae bacteria into the right gluteal region, inflammatory symptoms (severe swelling, erythema, and reduced mobility) developed within the first 2 days in the knee, ankle, and phalangeal joints, surrounding muscles and tendons of the right hind leg and, 2 days later, of the left hind leg (Fig. 1). The local inflammatory symptoms were paralleled by a severe loss in body weight that began at day 2 after infection. Until day 5 a reduction of 25 to 35% of body weight was noted when compared with noninfected animals (Fig. 1). Furthermore, 3 days after infection, we observed general symptoms of inflammation, such as lethargy, fever, and ruffled fur. Frequently, a terminal tail necrosis was noted.

Localization of injected bacteria. To follow the body distribution of intramuscularly injected bacteria, E. rhusiopathiae was labeled with ⁷⁵Se and radioactivity was measured in various organs on days 4 and 11 after infection (Fig. 2). The highest ⁷⁵Se activity was found in the liver, followed by the kidneys and blood. The spleen, lung, and both knee joints contained rather little activity, and the original gluteal injection site was almost free from radioactive label. Since at day 4 only a few and at day 11 no viable bacteria could be cultured from organ homogenates or blood, it appears likely that recovered radioactivity represents a distribution of bacterial breakdown products. This assumption was supported by microscopic studies, demonstrating a predominant deposition of bacteria in Kupffer cells of the liver and, to a lesser degree, in glomerula (Rüschoff et al., manuscript in preparation). The crucial finding was that the knee joints, most severely and persistently affected after E. rhusiopathiae infection, did not accumulate enhanced levels of radioactivity.

Release of TNF-α, IL-1, and PGE₂. The dramatic loss in body weight, together with symptoms of inflammation, suggested that conspicuous arthritis was accompanied by a more general alteration of the immune system. Since the macrophage cytokines TNF-α and IL-1 and the arachidonic acid metabolite PGE₂ have previously been implicated in inducing inflammation (3, 9, 12, 33), we examined the release kinetics of these mediators. We had to restrict ourselves to the easily accessible peritoneal macrophages, since attempts to isolate a sufficient number of cells from the small-sized inflamed joints of rats were unsuccessful. When the acute phase until day 11 post-E. rhusiopathiae infection was

FIG. 1. Development of hind leg arthritis and loss of body weight in Lewis rats intraglutely injected with 10⁴ E. rhusiopathiae (E. rhus.) bacteria at day 0. Each time point represents the mean ± standard deviation of a group of five animals.

FIG. 2. Distribution of ⁷⁵Se-labeled E. rhusiopathiae (E.r.). A total amount of 200 × 10⁵ cpm was injected intraglutely on day 0, and recovery in various body compartments is shown on days 4 and 11 after infection.
examined, we observed a diversified pattern of mediator release (Fig. 3). The earliest cytokine was TNF-α, which was spontaneously released from macrophages in extremely large amounts on day 2 and after this singular peak declined but remained elevated on the following days. Attempts to detect enhanced levels of TNF-α in serum failed, which may be explained by the short half-life of this cytokine in circulation (4, 32). The peak of IL-1 production was found on day 3, and thereafter, a decline to near-normal levels occurred. In contrast to these cytokines, an enhanced release of PGE₂ did not start before 3 to 4 days after *E. rhusiopathiae* infection but then continuously increased up to day 11.

![Graph](Image)

**FIG. 3.** Release of PGE₂, TNF-α, and IL-1 from macrophages at different times after *E. rhusiopathiae* infection. Peritoneal macrophages were obtained on the indicated days and incubated at a concentration of 0.5 x 10⁶/ml. After 20 h of incubation, the culture supernatants were harvested and release of PGE₂, TNF-α, and IL-1 was determined. The values represent the mean ± standard deviation of macrophages from three rats per time point.

**TABLE 1.** Effect of in vivo indomethacin treatment on TNF-α release from peritoneal macrophages of *E. rhusiopathiae*-infected rats

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>TNF-α (pg/ml)</th>
<th>Indomethacin</th>
<th>+ Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;2.5</td>
<td>1.440 ± 75</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.0 ± 0.6</td>
<td>1.11 ± 3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>13,430 ± 1,750</td>
<td>14,380 ± 1,100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14 ± 0.9</td>
<td>111 ± 5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11 ± 0.6</td>
<td>25 ± 3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>&lt;2.5</td>
<td>258 ± 21</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>276 ± 18</td>
<td>1,920 ± 140</td>
<td></td>
</tr>
</tbody>
</table>

* Peritoneal macrophages were harvested at the indicated times from *E. rhusiopathiae*-infected rats with or without continuous indomethacin treatment (10 mg/kg per day). Peritoneal macrophages (0.5 x 10⁶/ml) were incubated for 20 h and, thereafter, culture supernatants were assayed for TNF-α release.

* Mean ± standard deviation of macrophages from three rats per time point.

When PGE₂ production was blocked by a continuous treatment of *E. rhusiopathiae*-infected rats with indomethacin, TNF-α release from macrophages occurred earlier and persisted at higher levels than in untreated animals (Table 1). Moreover, indomethacin produced a more rapid weight loss and lethality in 70 to 80% of rats with an otherwise tolerable dose of 10⁶ *E. rhusiopathiae* bacteria. Thus, an inverse relationship may exist between both mediators, in that an initially enhanced TNF-α release may stimulate PGE₂ production, which in turn may inhibit further TNF-α synthesis (6, 26, 35).

A comparison of TNF-α release from peritoneal macrophages with that of mesenteric lymph nodes and spleen revealed that lymph node cells were particularly responsive to *E. rhusiopathiae* infection and almost immediately released elevated levels of TNF-α on day 1, whereas spleen cells displayed a response pattern similar to that of peritoneal macrophages (Table 2). With both organ cells a singular peak of TNF-α release was observed, which was rapidly followed by a decline to normal levels. The available bioassay did not allow us to differentiate whether the released product was TNF-α or the lymphocyte product lymphotixin (TNF-β).

To address the question of whether *E. rhusiopathiae* bacteria would directly stimulate TNF-α and PGE₂ release, resident peritoneal macrophages were incubated in vitro with viable or sonicated *E. rhusiopathiae* (Table 3). Viable *E. rhusiopathiae* organisms stimulated a high TNF-α release but only a moderate PGE₂ release, whereas sonicated *E. rhusiopathiae* induced only TNF-α but not PGE₂ synthesis.

**TABLE 2.** TNF-α release from peritoneal macrophages, lymph node cells, and spleen cells of *E. rhusiopathiae*-infected rats

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>TNF-α (pg/ml)</th>
<th>Macrophages</th>
<th>Lymph node cells</th>
<th>Spleen cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.1</td>
<td>0.6 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.0 ± 0.8</td>
<td>2,200 ± 125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>27,000 ± 2,650</td>
<td>215 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14,8 ± 2.2</td>
<td>2.4 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.2 ± 1.5</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.3 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>620 ± 35</td>
<td>&lt;0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Cells were harvested at the indicated times after *E. rhusiopathiae* infection. Peritoneal macrophages (0.5 x 10⁶/ml), lymph node cells (1 x 10⁶/ml), and spleen cells (1 x 10⁶/ml) were incubated for 20 h, and culture supernatants were assayed for TNF-α release.

* Mean ± standard deviation of three rats per time point.

**TABLE 3.** In vitro stimulation of TNF-α and PGE₂ release from macrophages by *E. rhusiopathiae* or *E. coli*

<table>
<thead>
<tr>
<th>Addition</th>
<th>TNF-α (pg/ml)</th>
<th>PGE₂ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>180 ± 19⁺</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td><em>E. rhusiopathiae</em>, viable, 10⁴</td>
<td>&gt;20,000</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td><em>E. rhusiopathiae</em>, sonicated, 10²</td>
<td>1,240 ± 175</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td><em>E. rhusiopathiae</em>, sonicated, 10³</td>
<td>2,200 ± 205</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td><em>E. rhusiopathiae</em>, sonicated, 10⁴</td>
<td>19,870 ± 3,150</td>
<td>15.1 ± 0.9</td>
</tr>
<tr>
<td><em>E. coli</em>, viable, 10⁴</td>
<td>&gt;20,000</td>
<td>18.3 ± 1.1</td>
</tr>
<tr>
<td><em>E. coli</em> LPS*, 1 µg/ml</td>
<td>&gt;20,000</td>
<td>15.1 ± 0.9</td>
</tr>
</tbody>
</table>

* Resistant peritoneal macrophages (0.5 x 10⁶/ml) were incubated without or with *E. rhusiopathiae* or *E. coli* preparations as indicated. After 20 h of incubation, culture supernatants were assayed for TNF-α and PGE₂.

* Mean ± standard deviation of triplicate incubations.

* LPS, Lipopolysaccharide.
In contrast, viable *E. coli* bacteria and *E. coli* lipopolysaccharide were both capable of inducing TNF-α and, in addition, PGE<sub>2</sub> production. This difference in PGE<sub>2</sub> production between *E. rhusiopathiae* and *E. coli* suggested that under in vivo conditions, PGE<sub>2</sub> and possibly also TNF-α release might not be solely due to a direct effect of *E. rhusiopathiae* bacteria on macrophages. To examine the participation of lymphocytes and lymphocyte products on macrophage activation to TNF-α and PGE<sub>2</sub> release, *E. rhusiopathiae*-infected rats were treated with CsA.

**Reduction of TNF-α and PGE<sub>2</sub> release by CsA.** Treatment of animals with CsA alleviated the general inflammatory symptoms such as ruffled fur and lethargy. Locally, there was less swelling and erythema of the joints, and mobility was partially maintained. The weight loss was only 15 to 20% when compared with healthy animals. CsA treatment of *E. rhusiopathiae*-infected rats had pronounced effects on TNF-α release from peritoneal macrophages (Fig. 4). The single peak of TNF-α production shifted from day 2 to day 5 and, in addition, the amount was markedly reduced to 1/10 of that TNF-α which was released from macrophages of untreated rats. A similarly delayed and reduced TNF-α secretion during CsA treatment was also found in spleen and lymph node cells (data not shown). The secretion of IL-1 during CsA treatment was less affected and resulted only in a reduction of 45%, without an apparent shift of maximal production to the right (data not shown). CsA also counteracted PGE<sub>2</sub> release from peritoneal macrophages and reduced PGE<sub>2</sub> levels to near normal until day 8, which was in marked contrast to the high PGE<sub>2</sub> production of infected but untreated rats (Fig. 5). The low TNF-α and PGE<sub>2</sub> release from macrophages of noninfected rats remained unaffected by CsA treatment.

**Stimulation of TNF-α release by lymphocyte supernatants.** In an attempt to clarify the role of lymphocyte products, resident peritoneal macrophages of healthy rats were incubated with 20-h culture supernatants that were collected from mesenteric lymph node cells obtained at various times after *E. rhusiopathiae* infection (Fig. 6). Incubation of macrophages with supernatants of lymphocytes collected on days 3 and 5 after infection led to a significant increase in TNF-α production. Although lymphocytes themselves released TNF on day 1 after infection (Table 1), they were additionally capable of releasing factors on the following days that activated macrophages to enhanced TNF-α production. Again, CsA treatment displayed a suppressive effect on lymphokine release in that the macrophage-activating capacity was strongly reduced on day 3 and was entirely abrogated on day 5. Unfortunately, the identity of the active lymphokine is still unclear. However, a number of experiments showed that it was not an interferon or IL-1 and it displayed heat lability when incubated for 30 min at 60°C.
Reduced *E. rhusiopathiae* susceptibility of T-cell-deficient rats. To further assess the possible contribution of T lymphocytes to *E. rhusiopathiae*-induced inflammation and arthritis, the reaction pattern of T-cell-deficient nude rats was examined (Table 4). Han-rnu rats were found to be strongly resistant to *E. rhusiopathiae* doses of lower than 10^6 bacteria. Furthermore, they displayed less severe inflammatory symptoms, had less body weight reduction, and most importantly, developed only moderately acute but not chronic arthritis.

**Proliferative response of spleen lymphocytes and thymocytes.** *E. rhusiopathiae* infection affected lymphocyte proliferation of the spleen and thymus in an entirely opposite manner (Fig. 7). Spleen lymphocyte stimulation by concanavalin A during the acute phase of *E. rhusiopathiae* infection was strongly suppressed but could be partially restored by the addition of the cyclooxygenase blocker indomethacin, which indicated that PGE2-producing suppressor macrophages (14, 27) may have downregulated lymphocyte proliferation. In sharp contrast, thymocytes, which normally display a rather reduced proliferation in response to concanavalin A, were found to exhibit a strongly enhanced mitogen response, indicating that *E. rhusiopathiae* infection had dramatic effects on the maturation of thymic lymphocytes.

**Induction of inflammation and arthritis by dead *E. rhusiopathiae* .** To address the question of whether bacteria had to be viable to initiate inflammation and acute and chronic arthritis, rats were intragastrically injected with sonication-killed *E. rhusiopathiae*. Table 5 summarizes the findings which clearly indicate that not only inflammatory symptoms but also, more importantly, both acute arthritis and chronic arthritis were caused by dead *E. rhusiopathiae*, albeit higher doses had to be employed.

**DISCUSSION**

In swine an acute infection with *E. rhusiopathiae* results in a chronic destructive arthritis which resembles, in many respects, human rheumatoid arthritis (43, 49, 50). After adaption into rats (31), an experimental model became available that enabled us to follow a sequence of events that may lead to chronic arthritis. This infection-induced model displays characteristics that may supplement other currently used experimental arthritis systems (19, 40-42, 45, 46). Although *E. rhusiopathiae* bacteria are rapidly cleared after an apparent septicemic phase of several days (50), the hind leg arthritis outlasts the acute inflammatory response and persists during the entire life span of the animal. The advantage of this experimental system is that it is triggered by a pathogen which is known to naturally cause chronic arthritis in swine. Thus, it may be a useful model to understand how some other suspected bacteria such as *Klebsiella, Shigella, Yersinia*, and *Campylobacter* species may be involved in human arthritis (46). In this report we characterize the acute phase of *E. rhusiopathiae*-induced arthritis; separate communications will address the chronic phase and the transfer of arthritis by T lymphocytes.

*E. rhusiopathiae* induced a rapid and generalized infection which was characterized by typical clinical symptoms such as fever, malaise, and lethargy. From the local gluteal injection area the bacteria appeared to spread rapidly (Fig. 2), and the bacterial label was found predominantly in the primary sequestration organ, the liver, but also in the kidneys and blood circulation. Although acute inflammatory symptoms developed in all joints of both hind legs between days 2 and 3 after infection, a preferential accumulation of bacteria in articular joints was not detected. The most marked clinical symptom was a dramatic weight loss which occurred rapidly on day 2 after infection and persisted for the entire observation period of 10 to 11 days (Fig. 1). Recent reports have clearly indicated that weight loss and cachexia represent a shift from energy storage to energy utilization mediated by the factor cachectin, which is identical to TNF-α (2, 3, 44). Since macrophages are the primary source of TNF-α, the above-described symptoms already pointed to macrophages as important effector cells of *E. rhusiopathiae*-induced inflammation.

The acute phase of *E. rhusiopathiae*-induced inflammation was clearly related to a systemic activation of macrophages.
This became evident by an enhanced release of TNF-α, IL-1, and PGE₂, which, as typical macrophage products, have long been established as mediators of inflammation (3, 9, 12, 33, 44). A particularly striking feature was the rapid onset of TNF-α release from lymph node cells on day 1 (Table 2) and from peritoneal macrophages and spleen cells on day 2 after infection (Fig. 3). This observation suggested that the spread of bacteria or bacterial degradation products may have directly induced TNF-α synthesis. This assumption was partly substantiated by the in vitro finding that E. rhusiopathiae, either viable or sonicated, was indeed capable of directly stimulating TNF-α release from macrophages (Table 3). Release of TNF-α and IL-1 was characterized by a singular peak on days 2 and 3 after infection, respectively, which indicates that either the stimulus was short-lived or appropriate down-regulating control mechanisms were induced. Similar observations with respect to only brief peaks of TNF-α production after lipopolysaccharide injection or during gram-negative sepsis have recently been reported (32), which are reconfirmed by our data, with the notable difference that a gram-positive bacterium has been examined. A potential TNF-α counteracting inhibitor may be PGE₂, which was released in a delayed fashion and increased in parallel to the decrease in TNF-α. PGE₂ release has been shown to be stimulated by TNF-α (6), and high concentrations of PGE₂, in turn, have been implicated in suppressing TNF-α synthesis (26, 35), which, taken together, could readily explain the temporally different appearance of both mediators during the acute phase of E. rhusiopathiae-induced inflammation. This assumption is further supported by our data (Table 1) showing that inhibition of prostaglandin synthesis by treatment of E. rhusiopathiae-infected animals with indomethacin enhanced and prolonged TNF-α release and, furthermore, aggravated the disease to a lethal outcome.

In addition to an apparently direct effect of E. rhusiopathiae on macrophages, evidence has also been obtained that lymphocytes and lymphocyte products were involved in macrophage activation to mediator release. We used CsA to suppress lymphocyte stimulation and lymphokine production (10, 48) and employed T-cell-deficient nude rats to test lymphocyte participation. Treatment of infected animals with CsA drastically reduced and delayed TNF-α and PGE₂ release and, in addition, inflammatory symptoms were less severe and the weight loss was not as dramatic as in untreated, infected animals. The role of soluble lymphocyte products became clearly evident when only the lymphocyte supernatants from infected but not those from infected and simultaneously CsA-treated animals were capable of stimulating TNF-α release from macrophages. Although our attempts to identify the exact biochemical nature of the active lymphokines have failed so far, the findings taken together clearly suggest that two effector pathways have led to an enhanced inflammatory mediator release from macrophages: a direct effect of bacterial products and an indirect effect mediated by lymphokines from sensitized lymphocytes.

The pivotal role of T lymphocytes during the acute inflammatory stage and for the transition into chronic arthritis was substantiated in nude rats (Table 4). With these T-cell-deficient animals a significantly higher dose of E. rhusiopathiae was required to establish an infection. Moreover, reduction in body weight, inflammation, and acute arthritis were less severe, which indirectly indicates that release of TNF-α and other mediators may have been lower. However, detailed studies must still be performed to provide evidence for this assumption. Of particular importance was the observation that in nude rats a transition from the acute to chronic joint inflammation did not occur, which underlines the notion that T cells are indispensable for the development of chronic arthritis in this model system.

The enhanced lymphokine release during the acute inflammatory response to E. rhusiopathiae (Fig. 6) was not associated with an augmented but, in contrast, with a suppressed mitogen response of spleen lymphocytes (Fig. 7). This finding points to a dichotomy of lymphocyte functions in that enhanced lymphokine release may not necessarily parallel elevated proliferation. As previously shown and discussed in detail (12, 14, 17), suppression of lymphocyte proliferation was partially due to activated macrophages which inhibited lymphocytes via an indomethacin-sensitive pathway. Most likely, these suppressor macrophages acted via an enhanced release of PGE₂, which has been previously established as the main macrophage product that inhibits lymphocyte proliferation. It appears that generation of suppressor macrophages is due to lymphokines which are a part of a feedback system that counterbalances an overshooting lymphocyte response (12). However, in this experimental model system, feedback inhibition systems may not be entirely operative as macrophage activation appears to persist in certain locations, such as the hind leg joints. This dysregulation leading to locally persistent macrophage activation may be attributable to lymphokines which are released continuously from stimulated lymphocytes. Further studies are required to determine whether the underlying cause of such continuous lymphocyte stimulation is due to a long-lasting persistence of bacterial antigen, a cross-reactivity of bacterial antigen with host components, or an infection-induced generation of autoreactive T cells.

In support for the pivotal role of certain T lymphocytes in chronic arthritis, we found that E. rhusiopathiae-induced inflammation had rapidly induced a progressive stage of thymocyte maturation which was manifested here by a markedly enhanced mitogen response (Fig. 7). A separate communication will describe the salient features of thymus morphology, rapid thymocyte maturation into single CD₄ and CD₈-positive subsets, and transferability of the disease by thymocytes (Renz et al., manuscript in preparation). Taken together, this animal model represents a valuable tool to examine not only an inflammatory response to bacterial infection but also, more important, the parallel induction and persistence of arthritis. In particular, it will allow studies directed toward dissecting interactions between macrophages and lymphocytes and, furthermore, defining lymphocyte subpopulations that maintain chronic arthritis.

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