Use of Lymphokines in Treatment of Experimental Intra-Abdominal Abscess Caused by Bacteroides fragilis

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The role of cell-free soluble factors (lymphokines) derived from mitogen-activated splenic cells of mice previously immunized against Bacteroides fragilis was evaluated in the treatment of B. fragilis intra-abdominal abscess (IAA). Neither clindamycin nor lymphokines alone were effective against an established B. fragilis IAA, but the combination of clindamycin and lymphokines decreased the abscess size and bacterial counts in the majority of animals. This suggests that the synergy of lymphokines with clindamycin effects cure of IAA caused by B. fragilis and that lymphokines might have an application as adjuncts to conventional antimicrobial therapy in this setting.

Bacteroides fragilis, an anaerobic, gram-negative bacterium, is capable of inducing abscess in its host (9, 18). Attempts to resolve or sterilize the abscess with antibiotic therapy alone have been unsuccessful (2, 6). Effective therapy often requires repeated surgical pus drainage procedures and antibiotic therapy (6). These considerations prompted the search for the immune mechanisms responsible for the prevention of abscess formation, a search that ultimately may lead to the development of noninvasive therapeutic strategies.

Recent research indicates that T lymphocytes play a protective role against the development of abscess caused by B. fragilis (12, 13, 15, 16). T cells appear to exert this protective effect by releasing soluble factors (lymphokines) (21). The soluble T-cell factors are effective in preventing experimental intra-abdominal abscess formation when used prophylactically (21). However, it is unclear whether lymphokines are also effective in promoting the clearance or sterilization of established abscesses. Therefore, we investigated the potential therapeutic efficacy of administering lymphokines in vivo either alone or in combination with the antibiotic clindamycin to clear established abscesses in an experimental mouse model.

MATERIALS AND METHODS

Preparation of lymphokines. Mice aged 6 to 8 weeks (C3H/HeN) were immunized by subcutaneous injections with heat-inactivated B. fragilis (10⁶ organisms) once a week for 3 weeks. On day 1 after the last injection, the mice were sacrificed, and a single-cell suspension of their spleen cells was prepared as described elsewhere (7). Spleen cells (10 × 10⁶/ml) derived from immunized and unimmunized animals were cultured separately with or without 25 μg of concanavalin A (Con A) per ml in a humidified CO₂ incubator at 37°C for 24 h. At the end of incubation, Con A (25 μg/ml) was added to control cultures, and the culture supernatants were harvested by centrifugation. Culture supernatants were filtered (pore size, 0.45 μm) and stored at −20°C until use. The crude culture supernatants thus obtained were thereafter termed lymphokines.

Bacterial challenge and experiments. Intra-abdominal abscesses in mice were induced by intraperitoneal injections of 0.1 ml of an inoculum containing 10⁸ CFU of B. fragilis, 50% autoclaved rat fecal contents, and 10% BaSO₄ as previously described, with minor modifications (17). The organism used in this study was a clinical isolate from a patient with intra-abdominal abscess. Before use, the B. fragilis isolate was passaged through the peritoneal cavity of mice. Rat feces was dried, crushed, mixed with a volume of sterile distilled water twice that of the feces, and autoclaved. The resultant slurry was autoclaved, and the fecal extracts were removed aseptically. The extracts were autoclaved and mixed with equal volumes of a 24-h broth culture of B. fragilis, preadjusted to contain 2 × 10⁸ CFU/ml plus 10% BaSO₄ (wt/vol). Preliminary studies have shown that intra-peritoneal injection of mice with this inoculum results in abscess development in 5 days. In control experiments, intraperitoneal injection of autoclaved fecal contents plus BaSO₄ did not cause abscess.

At 5 to 7 days postinoculation, the mice were treated with lymphokines, clindamycin, or lymphokines plus clindamycin twice daily at 8-h intervals for 7 days. The doses of clindamycin were 75 mg/kg of body weight and were given subcutaneously. The amounts of lymphokine administered varied from 0.2 to 0.6 ml and were given intraperitoneally.

Assessment of results. Mice were killed 1 day after completion of therapy. Their abdomens were opened and examined for the presence or absence of abscesses. If abscesses were present, then pus was cultured to confirm the presence of B. fragilis. In this study, elimination of abscesses was considered a cure. A mere decline in number of organisms in the abscesses or size of the abscesses was not considered adequate to be called a cure. Results were analyzed for statistical significance by chi-square analysis, using the BMDP statistical software package. P values of <0.05 were considered significant.

Determination of viable B. fragilis in abscesses. Mice were killed 1 day after termination of therapy, their intra-abdominal abscesses were removed aseptically, and abscesses from each group were weighed and homogenized in a sterile tissue grinder that contained 1 ml of sterile saline. After thorough homogenization, the homogenates were serially diluted (10-fold dilutions) in sterile saline, and appropriate dilutions were plated on brain heart infusion blood agar plates supplemented with vitamin K and hemin. The plates were incubated at 37°C for 72 h in an anaerobic gas jar. Colonies...
TABLE 1. Therapeutic efficacy of lymphokines in clearing intra-abdominal abscesses caused by B. fragilis

<table>
<thead>
<tr>
<th>Groupa</th>
<th>Mouse treatment (amt)</th>
<th>No. of posttreatment mice:</th>
<th>% Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without abscess</td>
<td>With abscessb</td>
</tr>
<tr>
<td>1</td>
<td>Saline</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Lymphokine (0.2 ml)</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Clindamycin (75 mg/kg)</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Lymphokine (0.2 ml) + clindamycin (75 mg/kg)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Control culture supernatant (0.2 ml) + clindamycin (75 mg/kg)</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

a Each group consisted of 10 mice. Mice were injected intraperitoneally with an abscess-inducing mixture containing B. fragilis, and therapy was begun 5 days postinoculation. The mice were treated as indicated twice daily at 8-h intervals for 7 days.

b Abscess pus grew B. fragilis upon culture.

c Lymphokines were prepared from spleen cells of mice immune to B. fragilis as described in Materials and Methods.

P was <0.001 compared with saline (group 1) and lymphokine controls (groups 2 and 5) by the Pearson chi-square test (X² = 6.67). P was <0.05 compared with clindamycin control by the Pearson chi-square test (X² = 3.8).

d Culture medium was derived from immune spleen cells cultured in the absence of Con A. Con A was added to this medium at the termination of culture.

were counted from plates containing 30 to 300 colonies per plate, and results were expressed as CFU per milliliter. The lower limit of detection by this method is 10³ CFU/ml. Data obtained were evaluated with the Student t test of independent means.

Measurement of antibiotic level. Clindamycin levels in serum and abscess contents were measured at 0.5, 1, 2, and 4 h after dose 3 of clindamycin (75 mg/kg). Two animals were used at each timed interval. The mice were exsanguinated by cutting their brachial plexuses. Intra-abdominal abscesses were removed, weighed, and homogenized in 1 ml of saline. Levels of clindamycin in serum and pus were determined by bioassay with B. subtilis (3). Duplicate measurements were made on each sample, and the average values were reported. The susceptibility of B. fragilis to clindamycin was determined by the agar dilution method as previously described (1).

RESULTS

Effect of lymphokines on clearance of abscesses caused by B. fragilis. The therapeutic outcome of treating mice with lymphokines derived from the spleen cells of mice immune to B. fragilis, with clindamycin, or with clindamycin plus lymphokines is shown in Table 1. In these experiments, mice were injected intraperitoneally with an abscess-inducing mixture containing B. fragilis, and treatment was initiated 5 days after injection with B. fragilis. Day 6 was selected for the initiation of therapy on the basis of preliminary experiments which showed that abscesses were well developed at this time. Treatment of mice with lymphokines alone or clindamycin alone did not result in elimination of abscesses (Table 1, groups 2 and 3). However, treatment of mice with lymphokines plus clindamycin resulted in clearance of abscess in 5 of 10 animals. This therapeutic response was significantly different from that of control groups of animals that did not receive this regimen. Cell-free culture supernatants derived from unstimulated immune spleen cells had no effect on the clearance of abscesses (Table 1, group 5).

The effect of lymphokines on abscess clearance was dose dependent (Fig. 1). Increasing the amount of lymphokine administered with clindamycin from 0.2 to 0.4 or 0.6 ml resulted in the elimination of abscesses in 80% of the animals. The differences between the groups of animals treated with 0.4 or 0.6 ml of lymphokine plus antibiotic and the controls treated with antibiotic plus control culture supernatants were highly significant (X², 9.89; P <0.01). Therapy with 0.4 to 0.6 ml of lymphokine alone did not result in abscess elimination (data not shown).

Cell-free culture supernatants derived from Con A-activated splenocytes of unimmunized mice did not clear abscesses when it was injected with clindamycin (Table 2).

Effect of lymphokines derived from immune cells on bacterial counts. The number of viable bacteria recovered from the abscesses of mice treated with lymphokines alone was essentially identical to the number of bacteria recovered from untreated controls (Table 3, group 2). There were significantly lower numbers of B. fragilis in abscesses from mice treated with lymphokines plus clindamycin compared with the number in either untreated controls (group 1) or controls treated with clindamycin alone (group 3). Although clindamycin therapy alone caused a significant decrease in the number of organisms recovered from the abscesses, this therapeutic regimen did not eliminate abscesses. The organism used in this study was found to be susceptible in vitro to

FIG. 1. Effect of lymphokine dose on therapeutic outcome. Mice (10 per group) were treated with clindamycin plus indicated amounts of lymphokine preparation for 7 days. Cure was determined by the presence or absence of abscesses. Supt., Supernatant.
clindamycin (MIC, 0.5 μg/ml), which achieved levels in serum and pus above its MIC. The levels of clindamycin in serum were 5 ± 1.5 μg/ml 30 min after a 75-mg/kg dose was given subcutaneously. At this dose, the levels in pus at 30 min were found to be 2 ± 0.53 μg/ml.

**DISCUSSION**

Recent reports indicate that prophylactic therapy with cytokines derived from either T cells or monocytes can prevent development of infection, lessen the bacterial burden and severity of infection, and provide protection against death from infection caused by bacteria in mice (4, 5, 19–21). In this study, we report the efficacy of delayed administration of cytokine preparation in clearing intra-abdominal *B. fragilis* infection in mice. The results presented here demonstrate that lymphokine preparations in conjunction with an antibiotic can effectively clear intra-abdominal abscesses caused by *B. fragilis*. This conclusion is based on the following findings: (i) therapy with lymphokine preparation alone did not clear abscesses when it was given 5 days postinfection of mice with *B. fragilis*, (ii) delayed treatment with clindamycin alone was also ineffective in sterilizing or resolving abscess, and (iii) when lymphokine preparations were given with clindamycin, there was a dramatic improvement in clearance of intra-abdominal abscesses.

Previous studies have demonstrated that soluble factors derived from the T cells of mice immune to *B. fragilis* can protect nonimmune animals when given 24 h prior to injection of *B. fragilis* (21). The results of this study indicate that optimum therapy for the treatment of established abscesses requires not only an immunomodulator but also an antibiotic that is active in vitro against *B. fragilis*.

The potentiation of abscess sterilization by cell-free culture supernatants in our study is probably not due to Con A, an immunomodulator (8, 11, 14), because control culture supernatants to which Con A was added were ineffective in clearing the abscess when they were injected with clindamycin (Table 1). These results suggest that splenocytes immune to *B. fragilis* release a soluble factor upon activation with Con A and that this cytokine is active in promoting the sterilization and elimination of abscesses.

Con A-activated spleen cells produce a wide variety of factors (cytokines) that possess potent immunomodulatory properties. One might have expected that it would be difficult to achieve identification of the cytokine responsible for the observed therapeutic efficacy in our study. While identification of the relevant cytokine(s) eludes us, it is worthwhile to reflect on the kinds of cytokines that can be excluded, in part, on the basis of the absence or presence of cytokines in control culture supernatants. Nonspecific factors are easy to identify because they are produced by nonimmune and immune splenocytes upon activation by Con A. Such factors can be excluded from consideration in that cell-free culture supernatants derived from mitogen-activated spleen cells of unimmunized mice did not clear abscess when administered with antibiotic. It is important to emphasize that this kind of analysis does not necessarily exclude a role for a nonspecific factor in the accelerated therapeutic response of an infected host. Such factors may be required but not sufficient to cause enhanced clearance of intra-abdominal abscesses caused by *B. fragilis*.

Of the specific factors released from the immune spleen cells, antibodies to *B. fragilis* can be excluded from consideration because unstimulated immune spleen cultures, which contained products of plasma cells (i.e., antibodies against *B. fragilis*), were inactive in clearing abscesses. Furthermore, it has been shown by other investigators that the antibodies against *B. fragilis* do not confer protection against development of intra-abdominal abscesses caused by *B. fragilis* in experimental animal models (15, 17).

Our current working hypothesis is that Con A activates *B. fragilis*-primed T cells to release a cytokine(s) which acts in concert with antibiotic to bring about the accelerated elimination of intra-abdominal abscesses. The nature of the cytokine and the specificity of the lymphokine to the infecting organism remain to be elucidated.

The failure of clindamycin to clear intra-abdominal abscess despite levels in serum and pus above its MIC against *B. fragilis* is not surprising when considered in the light of

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (dosage [BID*])</th>
<th>Lymphokine source</th>
<th>No. of mice with abscess/total no. of mice</th>
<th>% Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lymphokine (0.4 ml) + clindamycin (75 mg/kg)</td>
<td><em>B. fragilis</em>-primed spleen</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Lymphokine (0.4 ml) + clindamycin (75 mg/kg)</td>
<td>Unimmunized spleen</td>
<td>5/5</td>
<td>0</td>
</tr>
</tbody>
</table>

* BID, Twice a day.
* P was <0.01 compared with group 3 by the Pearson chi-square test (χ² = 6.66).

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**TABLE 3. Effect of lymphokines on bacterial counts**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mouse treatment (amt)</th>
<th>No. of mice with abscess/total no. of mice</th>
<th>Total wt of abscess (mg)</th>
<th>CFU of <em>B. fragilis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Per ml (mean ± SD)</td>
</tr>
<tr>
<td>1</td>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lymphokine* (0.2 ml)</td>
<td>5/5</td>
<td>422</td>
<td>1.59 x 10⁸ ± 0.7 x 1⁰</td>
</tr>
<tr>
<td>3</td>
<td>Clindamycin (75 mg/kg)</td>
<td>4/5</td>
<td>396.3</td>
<td>1.56 x 10⁸ ± 0.07 x 1⁰</td>
</tr>
<tr>
<td>4</td>
<td>Lymphokine (0.2 ml) + clindamycin (75 mg/kg)</td>
<td>2/5</td>
<td>251.5</td>
<td>1.86 x 10⁹ ± 0.09 x 1⁰</td>
</tr>
</tbody>
</table>

* Derived from Con A-activated spleen cells of mice immune to *B. fragilis*.
* P was <0.05 compared with group 1 by the Student t test.
* P was <0.001 compared with groups 1 and 3 by the Student t test. Pus was obtained from abscesses of mice that were not cured by immuno-adjunct therapy.

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the reports of Bartlett et al. (2) and Joiner et al. (10). These authors demonstrated that the time of administration of clindamycin in relation to the time of infection is critical to the outcome: a progressive delay in the treatment was found to be associated with a decline in in vivo activity of the drug. In this study we have demonstrated that the drug failure associated with delayed treatment can be rectified by cytokines derived from spleen cells of mice immune to *B. fragilis*.

In view of the absence of any detectable bactericidal effects of cytokines derived from immune spleen cells on *B. fragilis* in vivo (Table 3, group 2), we believe that the most likely explanation for our results is one of the following: (i) cytokines potentiate the antibacterial effect of clindamycin, or (ii) cytokines enhance antibacterial cellular defense mechanisms in the host. Experiments are in progress to determine which of the aforementioned hypotheses is correct.

In summary, the results of this study imply that immunoadjuvant therapy might be of value in the treatment of *B. fragilis* infections that are difficult to cure by antibiotic therapy alone.

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LITERATURE CITED


