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Enhanced Protection by Use of a Combination of Anticapsole and Antilipopolysaccharide Monoclonal Antibodies against Lethal Escherichia coli O18K5 Infection of Mice

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Received 1 May 1995/Returned for modification 23 June 1995/Accepted 13 December 1995

To study antibody-mediated protection against Escherichia coli peritonitis in BALB/c mice, monoclonal antibodies (MAbs) were generated against the capsule (K5) and the lipopolysaccharide (O18) of E. coli. Flow cytometric analysis with two selected immunoglobulin M MAbs revealed that bacteria were antigenically heterogeneous. Arbitrarily, three subpopulations in E. coli O18K5 cultures could be distinguished by double immunofluorescence. A subpopulation bound only the anti-K5 MAb, and another subpopulation bound only the anti-O18 MAb. An intermediate subpopulation, however, bound both MAbs. In agreement with this result, combinations of both MAbs enhanced phagocytosis of fluorescein isothiocyanate-labeled bacteria by human polymorphonuclear leukocytes and mouse macrophage J774 cells as well. In protection experiments, combinations of both MAbs enhanced survival in mice. Protection of mice by the combination of MAbs was associated with significantly lower (P < 0.01) mortality in comparison with any other treatment group. Similarly, prophylactic administration of MAbs yielded significantly lower (P < 0.01) mortality factor levels in serum 90 min after challenge compared with any other treatment group.

Despite potent antibiotics, gram-negative bacteremia and sepsis remain a frequent cause of death among hospitalized patients (33, 34). Escherichia coli strains are most frequently isolated from these patients (12, 19). The development of protective antibodies for passive immunization against gram-negative bacteria is therefore an important objective. Lipopolysaccharide (LPS), a constituent of the outer membrane of gram-negative bacteria, consists of lipid A (the toxic moiety of LPS), a relatively conserved core oligosaccharide, and a terminal polysaccharide of variable length and composition that comprises the O-antigen-specific domain. Effort has been invested in the development of monoclonal antibodies (MAbs) against lipid A, the toxic moiety of LPS (endotoxin), which is highly conserved among gram-negative bacteria. In contrast to capsules and O antigens, lipid A is not highly variable, and cross-reactive lipid A antibodies have been demonstrated. So far, however, no conclusive data which show a protective activity of these cross-reactive antibodies in patients have been obtained (13, 35). Therefore, it may be worthwhile to develop MAbs against the capsules and O antigens of the most prevalent virulent strains of E. coli. Such MAbs have proven to be protective in animal models (7, 23, 25, 28). Enhanced protection by the combination of anti-K1 and anti-O18 MAbs against E. coli O18K1 has been described (17). However, the mechanism of the additional protection has not been elucidated.

In this study, we observed a similar enhanced protection for the combination of anticapsole and anti-O MAbs for E. coli O18K5. The O18 antigen is one of the four most prevalent O antigens of E. coli strains isolated from bacteremic patients (3, 10, 18). The immunogenicity of the K5 capsule is relatively low, which might be due to the similarity of the capsular polysaccharide and the host carbohydrates (6, 15). Therefore, MAbs of the indicated specificities could be of value as prophylactic and/or therapeutic agents.

The binding of anti-O18 and anti-K5 MAbs to E. coli O18K5 was examined by using an enzyme-linked immunosorbent assay (ELISA) and flow cytometry. Functional activities of MAbs were studied by in vitro phagocytosis and in vivo protection. Before intraperitoneal (i.p.) challenge of mice, the bacteria were preincubated with MAbs. This procedure enabled comparison of in vitro binding of MAbs with protection results. Mice protection studies were also performed with prophylactically given MAbs.

MATERIALS AND METHODS

Bacterial strains. The E. coli strains used in this study (O18K5, O18K1, O18K1, O2K5, and O1K1) were all clinical isolates from the Utrecht University Hospital and have been previously described (10). The bacteria were cultured at 37°C overnight either on blood agar plates or in Mueller-Hinton broth. Bacteria scraped from agar plates or centrifuged material collected from broth were spectrophotometrically adjusted in phosphate-buffered saline (PBS; pH 7.2) to an optical density that corresponded to a concentration of 2.5 × 10⁸ bacteria per ml. MAbs, MAbs were generated against the K5 capsule and O18 LPS of E. coli strains. Two independent immunization schedules were followed. BALB/c mice were injected i.p. with 10⁹ gentamicin-treated bacteria from pools of several clinical E. coli isolates (either the O18K or K5 serotype) four times at weekly intervals. A week after the last injection, sera obtained from ether-anesthetized mice were tested for the presence of antibodies against several O18K or K5 E. coli isolates. When no agglutination of bacteria was observed, immunization was continued for another 4 weeks, using the same protocol. For the O18K immunization, still another 4 weeks of immunization was necessary to obtain antibodies against all of the O18K isolates.

After a 1-month rest, an intravenous (i.v.) booster of a mixture of three clinical isolates of the desired serotype containing 10⁸ gentamicin-treated bacteria was given. Splenocytes were collected 3 days after the booster and fused with the SP2/0 myeloma cell line, using electrofusion (31). A fusion pulse of either 2 or 3
kV/cm was given with a duration of 10 μs. Hybridoma supernatant fluids were assayed by agglutination to detect the presence of specific antibodies against either E. coli O15K— or E. coli K5. A hybridoma (98.65) producing MAbs that reacted with O18 isolates and a hybridoma (91.71) producing antibodies to K5 isolates were then selected. Both hybridomas produced MAbs of the immunoglobulin (IgM) isotype, as determined by their type-specific ELISA. The amount of IgM in ascitic fluid was determined by using the same ELISA with a standard IgM preparation. Anti-Helicobacter IgM and anti-Campylobacter MAbs (unpublished results) were used as control MAbs. Ascitic fluid was used in both ELISA and animal protection studies. Purified serum-free MAbs were used for flow cytometry.

To obtain purified MAbs, hybridomas were cultured in protein-free medium without additional nutrients (Gibco BRL/Life Technologies, Gaithersburg, Md.) (30). MAbs in hybridoma supernatants were precipitated by addition of 1 mol of MAb with 100 μl of FITC (1 mg/ml) dissolved in 0.1 M NaHCO3 (pH 9.6) and rotating the mixture in the dark for 1 h. Subsequently, the mixtures were dialyzed against double-distilled water for 1 h and overnight against PBS.

ELISA. Each well of ELISA plates (Titertek, Flow Laboratories, McLean, Va.) was coated with 100 μl of a suspension of 2.5 × 106 CFU of bacteria per ml in PBS (pH 7.2). The plates were incubated for 1 h at 37°C and kept overnight at 4°C. The plates were then washed five times with water containing 0.05% Tween 20. 100 μl of water containing 0.05% Tween 20, 50 μl of diluted MAb were added to each well. The plates were incubated at 37°C for 1 h and then washed five times with water containing 0.05% Tween 20. After a 1-h incubation at 37°C and five washings with water-Tween 20, the plates were treated with 0.1 ml of PBS with 1% BSA and 0.05% Tween 20 for 1 h. After incubation with biotinylated goat anti-mouse IgM (IgM) isotype, as determined by an isotype-specific ELISA. The amount of IgM in ascitic fluid was used as a measure for both uptake and binding of bacteria by PMN and referred to as phagocytosis. The mean fluorescence ratio was calculated to compare experiments from different days with each other; the mean fluorescence ratio was determined with human PMN or J774 cells coinoculated with MAbs was divided by the mean fluorescence of cells without MAbs.

Mice. Female BALB/c mice, 6 to 8 weeks old, were obtained from the Utrecht University animal facilities.

Animal protection studies. To determine the 50% lethal dose (LD50) of E. coli O18K5, five groups of six mice were inoculated i.p. with either 107, 108, 109, or 1010 CFU of E. coli O18K5 in 0.5 ml of PBS (pH 7.2). The LD50 was calculated by the method of Reed and Muench (27) and shown to be 2 × 108 CFU/ml. For protection experiments, mice were inoculated with 0.5 ml of a mixture of MAbs and 3 LD50s of E. coli O18K5. Before administration, this mixture was preincubated for 30 min at 37°C. Prophylactically, equal doses of MAbs were administered i.p. and i.v. 1 h before i.p. injection of 3 LD50s of E. coli O18K5. Deaths occurring generally within the 24-h survival period were recorded for 7 days.

Determination of TNF levels. Blood was obtained from ether-anesthetized mice by orbital puncture 90 min after challenge, and serum tumor necrosis factor (TNF) levels were determined by using the TNF-sensitive murine fibroblast cell line L929. Logarithmically growing L cells were treated with trypsin, suspended in Dulbecco’s modified Eagle’s medium with fetal calf serum, and seeded in microtiter wells of 96-well plates at a density of 2 × 104 cells per 100 μl, and incubated for 18 h at 37°C. Samples of TNF-containing sera were serially diluted (1/10, 1/100, 1/1000, 1/10,000, and 1/100,000) in Dulbecco’s modified Eagle’s medium-fetal calf serum supplemented with actinomycin D to a final concentration of 2 μM. L-cell monolayers incubated with medium only were used as controls for unlysed (0%) and completely lysed (100%) cells. Complete lysis was established by treating cells with 800 pg of human TNF per ml. A human TNF-a standard (National Institute for Biological Standards and Control, Hertfordshire, England) was included. After an 18-h incubation period, the plates were fixed for 15 min with 25% glutaraldehyde and washed with water. The residual cells were then stained with methyl blue in D staining solution. The TNF content (picograms per milliliter) for each sample was also calculated.

Statistical analysis. The chi-square test was applied to determine significant differences in survival between treatment groups. The Mann-Whitney test was used to determine significant differences in TNF levels among mice from different treatment groups.

RESULTS

Antigen binding activity. The specificities of the anti-K5 and anti-O18 MAbs were determined with ELISA (results not shown). Serial dilutions of each IgM MAb were tested on ELISA plates coated with E. coli O1K1, O1K1, O2K5, or O1K5. The reactivity of the anti-K5 and anti-O18 MAbs to E. coli O1K1 did not differ from that of the negative control MAb. The anti-O18 MAb bound very well to E. coli O1K1. In contrast, the reactivity of the anti-K5 MAb against E. coli O1K1 did not differ from that of the negative control MAb. A similar result was obtained for the anti-K5 MAb against E. coli O2K5. Thus, the anti-K5 MAb reacts only with the K5 capsule and the anti-O18 MAb reacts only with the O18 antigen.

To investigate whether the anti-K5 and anti-O18 MAbs detect the same individual E. coli O18K5 cell, double-immunofluorescence experiments were performed. To demonstrate double binding, one MAb was directly labeled with FITC and the other was detected by using a PE-labeled goat anti-mouse Ig antibody. Fig. E. coli O18K5 from a mixed infection was incubated sequentially with the two different MAbs. Double-fluorescing bacteria indicated binding of both MAbs to individual bacteria (Fig. 1A). Arbitrarily by immunofluorescence, three subpopulations of bacteria could be distinguished: one population that only
FIG. 1. Dot plots of double-immunofluorescence fluorescence-activated cell sorter analysis. (A) Dot plot of E. coli O18K5. The bacteria were sequentially incubated with the anti-O18 MAb and PE-labeled goat anti-mouse Ig and with the FITC-labeled anti-K5 MAb. (B to E) The indicated E. coli strains were used to test the specificity of anti-K5 and anti-O18 MAb binding. PE-labeled goat anti-mouse Ig was used to demonstrate anti-K5 MAb binding. The anti-O18 MAb was directly labeled with FITC. Quadrant boundaries were constructed on the basis of control incubations, with gelatin Hanks’ balanced salt solution and PE-labeled goat anti-mouse Ig.

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bound the anti-O18 MAb, one intermediate population that bound both MAbs, and one population that bound the anti-K5 MAb. The results also suggested a minor population to which neither MAb bound. In Table 1, the percentages of the different populations that could be discriminated by flow cytometry are shown for three consecutive experiments. In experiments 1 and 2, the anti-K5 MAb stained the largest proportion of the bacterial population, while in experiment 3, the population of double-stained bacteria was the greatest. As a control for non-specific staining in each experiment, several serotypes of E. coli strains were tested. E. coli O1K1, O18K1, O18K−, and O2K5 were sequentially incubated with the anti-K5 MAb and a PE-labeled goat anti-mouse Ig and then stained with the FITC-labeled anti-O18 MAb (Fig. 1B to E). No double fluorescence was detected for E. coli O1K1, O18K1, and O18K−, indicating again the specificity of the anti-O18 MAb for the O18 antigen and the specificity of the anti-K5 MAb for the K5 capsule. However, 10% of the E. coli O2K5 cells showed double immunofluorescence (Fig. 1E). This could be due to the fact that the strains were first incubated with the anti-K5 MAb, which could induce agglutination of the strains. Thus, double immunofluorescence indicated that by use of both MAbs, the majority (94%) of the E. coli O18K5 cells were stained, whereas this was not the case with either MAb alone.

Enhancement of phagocytosis by combinations of anti-K5 and anti-O18 MAbs. FITC-labeled bacteria were used to study the ability of purified anti-K5 and anti-O18 MAbs, either alone or in combination, to mediate phagocytosis of E. coli O18K5. These bacteria were opsonized with different concentrations of the purified IgM MAbs, and human or mouse pooled serum was added as a complement source. The PMN- or J774 cell-associated fluorescence was determined by flow cytometry. Figure 2A shows phagocytosis of E. coli O18K5 by human PMN. Either the anti-O18 or the anti-K5 MAb alone was able to enhance phagocytosis, although the anti-O18 MAb induced high phagocytosis at low concentrations and anti-K5 MAb was effective at higher concentrations. The combination of the anti-O18 and anti-K5 MAbs, however, enhanced phagocytosis by human PMN at a total MAb concentration of 10 μg/ml (P < 0.02) compared with each MAb alone. Phagocytosis could not be measured when heat-inactivated serum was used (data not shown). Comparable results were obtained with E. coli O18K5 bacteria grown for 4 h in Mueller-Hinton broth (data not shown). This at least additive enhancement of phagocytosis for the combination compared with each MAb alone was also measured when mouse macrophage J774 cells were used (Fig. 2B), although this difference did not reach statistical significance.

Mouse protection studies. The protective efficacy of the anti-K5 and anti-O18 MAbs, alone and in combination, was evaluated in a mouse infection model in which a MAb and three LD_{50}s of E. coli O18K5 (6 × 10^7 CFU) were incubated for 30 min at 37°C prior to i.p. injection. The survival rates in three consecutive experiments are given in Table 2. In experiment 3, serum was obtained to determine TNF 90 min after challenge; the results of the TNF determination are included in Table 2. The summarized survival rates of animals receiving either MAb alone were 4 of 18 (22%) and 1 of 18 (6%) for the anti-K5 and anti-O18 MAbs, respectively. The combination of two MAbs together, however, had a dramatic impact on survival: 100% of all infected animals survived, which corresponds to a significant reduction in mortality when compared with that observed with the anti-K5 MAb (P < 0.001) and the anti-O18 MAb (P < 0.0001). Only two animals in the PBS control group and four in the control (anti-Helicobacter) MAb group survived.

In experiment 3, the mean TNF levels in serum correlated with the mortality in the experiment (r = 0.84) as well as with the cumulative mortality in every group (r = 0.93). As also shown in Table 2, only the mice that had received the combination of MAbs had relatively low levels of TNF in serum, even though these mice received a smaller amount of MAbs than did mice in other treatment groups. Moreover, the observed values are at least 20-fold lower (P < 0.02) than those from any other treatment group. In addition, we performed protection experiments in which MAbs were administered independently of the bacteria. Prophylactically, MAbs were given both i.p. and i.v., and 1 h later, E. coli O18K5 (3 LD_{50}s) was injected by the i.p. route (Table 3). In this experiment, both the anti-K5 MAb alone and the combination of the anti-O18 and anti-K5 MAbs produced survival of all of the mice. However, the mice that received the combination of the anti-O18 and anti-K5 MAbs had the lowest TNF levels of the groups tested.

**TABLE 1.** Discrimination between different subpopulations of E. coli O18K5 by double immunofluorescence

<table>
<thead>
<tr>
<th>Binding to E. coli O18K5</th>
<th>Subpopulations (%)</th>
<th>Expt 1</th>
<th>Expt 2</th>
<th>Expt 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only anti-K5 MAb</td>
<td></td>
<td>53</td>
<td>46</td>
<td>34</td>
</tr>
<tr>
<td>Only anti-O18 MAb</td>
<td></td>
<td>4</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Both anti-K5 and anti-O18 MAbs</td>
<td></td>
<td>39</td>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>Neither MAb</td>
<td></td>
<td>4</td>
<td>18</td>
<td>6</td>
</tr>
</tbody>
</table>

* Results from three consecutive experiments are shown.

**DISCUSSION**

This study describes the enhanced protection against an otherwise lethal i.p. challenge of mice with E. coli O18K5 by two IgM MAbs, one directed against LPS (O18) and the other directed against the capsule (K5). The specificities of these MAbs were demonstrated by ELISA (results not shown) and flow cytometry (Fig. 1). The anti-O18 MAb bound only to O18 serotypes, and the anti-K5 MAb bound only to E. coli containing the K5 capsule.

Depending on environment and phase of growth, bacteria produce different amounts of capsular polysaccharides (32). Using flow cytometry (11), we also found gradually different bindings of the anti-O18 and anti-K5 MAbs to the O18K5 strain, as indicated by single- and double-stained bacteria. Arbitrarily, three major antigenically different subpopulations (phenotypes) could be distinguished in E. coli O18K5 from blood agar plates. One of the subpopulations consisted of bacteria that seemingly bound exclusively the anti-O18 MAb and thus produced no or a very little amount of the K5 capsule. Alternatively, the epitope recognized by the anti-K5 MAb may be lost by a reduction in length of the repeating sugar units constituting the capsule. Another subpopulation of bacteria bound only the anti-K5 MAb, suggesting that they had a lot of capsule, which prevented the anti-O18 MAb from binding to the O18 LPS beneath the capsule. A third subpopulation of bacteria (53%; Fig. 1A) possessed an intermediate amount of capsule because both MAbs could bind to these bacteria. There was, however, always a small percentage (about 6%; Fig. 1A) of unstained particles which could be explained by electronic background noise, caused by submicrometer particles in the sheath fluid or scatters from optical components (22). Alternatively, these unstained particles represent either mutants of the smooth O18 strain or serological variants of O18.
FIG. 2. The mean fluorescence ratios of human PMN (A) or mouse macrophage J774 cells (B) were obtained by phagocytosis of FITC-labeled E. coli O18K5 opsonized with different concentrations of MAbs in the presence of complement. Each bar represents the mean ratio ± the standard error of the mean of three consecutive experiments. GHBSS, gelatin Hanks' buffered salt solution.
TABLE 2. Determination of the protective capacity of an anti-K5 MAb and an anti-O18 MAb, either alone or in combination

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Specificity</th>
<th>Dose (μg/mouse)</th>
<th>No. surviving&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cumulative survival (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mean TNF level in serum (ng/ml) (range)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Expt 1</td>
<td>Expt 2</td>
<td>Expt 3</td>
<td></td>
</tr>
<tr>
<td>No MAb</td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2 (11)</td>
</tr>
<tr>
<td>Helicobacter sp.</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K5 capsule</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>O18 LPS</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O18K5</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K5 and O18</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> MAbs were preincubated for 30 min at 37°C with 6 × 10<sup>7</sup> CFU of E. coli O18K5 (3 LD<sub>50</sub>) before the mixture was injected i.p. into BALB/c mice. Survival was recorded for 7 days in each experiment.

<sup>b</sup> Six mice per group.

<sup>c</sup> Eighteen mice per group.

<sup>d</sup> Blood was obtained by retro-orbital puncture performed at 90 min after infection from every mouse in experiment 3.

<sup>e</sup> Mortality significantly less (P < 0.001) than that of groups receiving only the anti-K5 MAb and significantly less (P < 0.001) than that recorded for groups treated with the anti-O18 MAb alone.

<sup>f</sup> TNF level significantly lower (P < 0.02) than in any other group.

Until now five serovar variants of O18, e.g., O18ac and O18ab (14), have been identified. Nevertheless, flow cytometric analysis of MAb binding to individual E. coli O18K5 cultures revealed distinct subpopulations of bacteria (heterogeneity).

Heterogeneity of bacterial cultures has also been described for E. coli O18K<sup>−</sup> strains. Two antigenically different subpopulations were detected with anti-O18 MAbs (20). Two distinct subpopulations of E. coli O26:B6 identified on the basis of differential binding by a core-reactive MAb have been described (8). Distinct phenotypes have also been identified for E. coli J5 (9), indicating heterogeneity for this strain. Thus, antigenic heterogeneity in bacterial cultures might be a phenomenon more common than generally appreciated.

In vitro phagocytosis of E. coli O18K5 opsonized with the anti-O18 MAb in combination with the anti-K5 MAb compared with either MAb alone mediated by mouse macrophage J774 cells or human PMN in the presence of complement was enhanced (Fig. 2). This result strongly suggests that the mechanism of the in vivo enhanced protection mediated by the anti-O18 MAb in combination with the anti-K5 MAb (Table 2) is complement-dependent, antibody-mediated phagocytosis of the bacteria.

Although 3 LD<sub>50</sub> of E. coli O18K5 turned out to contain 6 × 10<sup>7</sup> bacteria, which is quite a large inoculum, this bacterial challenge is comparable to bacterial bolus infusions used in other animal models of infections (6). Furthermore, the protection experiments suggest that both the K5 capsule and the O18 LPS contribute to the virulence of E. coli O18K5. In agreement with this suggestion, Cross et al. (4) found that the LD<sub>50</sub> of the O18K5-encapsulated parent was similar to that of its unencapsulated mutant. Both the K5 capsule and the O18 LPS are presumably capable of preventing antibody-independent classical pathway activation by shielding deeper structures of the cell membrane that are able to activate this pathway (16, 24).

The protective capacity of anti-O18 antibodies has been described previously for neonatal rats (21, 23, 28). However, in neonatal rats, the immune system has not been fully developed, while we used mice at ages of between 6 and 8 weeks with a normally developed immune system. Our results are in agreement with those of Kim et al. (17), who described the enhanced protective efficacy of anti-K1 and anti-O18 MAbs in a rat O18K1 infection model. A total of 79% survived in the group receiving the combination, whereas only 25% survived in the groups receiving either MAb alone. Kim et al. (17) described enhanced phagocytosis of E. coli O18K1 mediated by either the anti-O18 or the anti-K1 MAb, in agreement with our results. However, the combination of both MAbs was not tested as we did in the present study.

The release of TNF in serum was measured 90 min after bacterial challenge, because it is known that 90 min after challenge the peak value is reached (6). Preincubation of the bacterial inoculum with the combination of the anti-O18 and anti-K5 MAbs inhibited the release of TNF in serum, while neither antibody alone did so (Table 2). The low TNF levels correlated with survival of the mice. The TNF levels in the group treated with both MAbs were lower than in the control groups. When prophylactically given the anti-O18 and anti-K5 MAbs, either alone or in combination, protected mice when the combination of the anti-O18 and anti-K5 MAbs was given but also when anti-K5 alone was given. However, prophylactically the combination of both MAbs significantly (P < 0.01) reduced the TNF levels compared with any other treatment group (Table 3). This means that the protective mechanism acted very quickly. Probably, the release of LPS was prevented by rapid phagocytosis so that priming of sensitive cells for TNF

TABLE 3. Prophylactic administration of anti-O18 and anti-K5 MAbs significantly reduced TNF levels upon bacterial challenge<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Specificity</th>
<th>Dose (μg/mouse)</th>
<th>Level of bacteremia</th>
<th>Mean CFU (10&lt;sup&gt;3&lt;/sup&gt;) ± SD/ml of serum</th>
<th>Mean TNF level in serum (ng/ml) ± SD&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. surviving&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campylobacter sp.</td>
<td>400</td>
<td>1</td>
<td>0.7 ± 0.6</td>
<td>18.6 ± 19.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>K5 capsule</td>
<td>400</td>
<td>5</td>
<td>9.6 ± 7.7</td>
<td>9.5 ± 6.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>O18 LPS</td>
<td>400</td>
<td>5</td>
<td>9.6 ± 7.7</td>
<td>9.5 ± 6.4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>K5 and O18</td>
<td>200 + 200</td>
<td>5</td>
<td>0.2 ± 0.3</td>
<td>0.5 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Equally divided doses of MAbs were administered to groups of six mice i.v. and i.p.; 1 h later, 6 × 10<sup>7</sup> CFU of E. coli O18K5 (3 LD<sub>50</sub>) was given i.p.

<sup>b</sup> Blood was obtained by retro-orbital puncture performed at 90 min after infection.

<sup>c</sup> TNF level significantly lower (P < 0.01) than in any other treatment group.
production could not reach a dangerously high level. Similar results, i.e., reduction of TNF after challenge with *E. coli* O18K1, were obtained with prophylactically administered combinations of anti-O18 and anti-K1 MAbs (our unpublished results).

Combination immunotherapy has also been described for other gram-negative bacteria. Cross et al. (5) used three antibodies to protect neutropenic rats against infection with *Pseudomonas aeruginosa*, which resulted in enhanced survival. The triple combination of a murine anti-*Pseudomonas* (IgG1) MAb, an anti-tumor NF-α MAB, and a rabbit polyclonal antiserum directed against J5 was most effective in this study.

In summary, the results of this study demonstrate that use of the combination of anti-K5 and anti-O18 MAbs resulted in significantly enhanced protection in our mouse model of bacterial peritonitis. Use of combinations of antiscapsule and anti-LPS antibodies might prevent selection of either capsule-rich or capsule-deficient bacteria in vivo. Escaping bacteria may cause more serious disease. Therefore, combinations of two (or more) antibodies to different cell wall components may be more beneficial in preventing and treating infection than use of antibodies with a single specificity.

REFERENCES


PROTECTION BY MAbs AGAINST *E. COlI*...