ENHANCEMENT OF THE HUMORAL IMMUNE RESPONSE AND RESISTANCE TO BACTERIAL INFECTION IN MICE BY THE ORAL ADMINISTRATION OF A BACTERIAL IMMUNOMODULATOR (OM-89).

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ABSTRACT

We investigated the effects of an Escherichia coli-derived product (OM-89) in mice. The oral administration of OM-89 led to a significant (p<0.05, Student’s t test) increase in the levels of IgA in intestinal secretions, which was at maximum 25 days after the end of the treatment, when a two-fold increase in IgA levels was observed. The i.p. inoculation of OM-89 induced the stimulation of anti-SRBC plaque-forming cells (PFC) in the spleen. The effect of OM-89 was dose-dependent and produced up to a 9-fold increase in PFC in the treated mice when compared to untreated controls. The oral administration of OM-89 proved to be effective in the enhancement of resistance to challenge i.p. inoculation with E.coli. 32% of OM-89-treated mice showed resistance to this experimental infection at minimal LD50. The combined effects of low environmental temperature and cyclophosphamide (CY) immunosuppression enabled us to enhance resistance towards Pseudomonas aeruginosa infection. The oral treatment with the immunomodulator induced a significant (p<0.05, Student’s t test) level of protection in CY-immunosuppressed mice to the intranasal infection with P. aeruginosa, when mice were kept at low environmental temperature right after the bacterial challenge. The protective effect of OM-89 treatment was dependent on both the environmental temperature and the timing of the experiment.

INTRODUCTION

Immunomodulating agents of bacterial origin have been found to either enhance or inhibit the functioning of immune cells (1,2,3,4). The behaviour of lymphocytes and macrophages may be significantly modified following the exposure to Gram positive and Gram negative bacteria, as well as to components of these organisms (5,6). It is therefore likely that these substances may influence the host’s resistance to infections.

The purpose of the present investigation was to study the effect of one of these agents, OM-89, an Escherichia coli-derived product, on the development of immune responses in mice.
mice, as well as on the modulation of resistance to different types of bacterial infections in both normal and immunocompromised mice.

EXPERIMENTAL PROCEDURES

Treatments With The Immunomodulator

Female Swiss mice (6-week old and weighing approx. 15-17 g; Biocentre, Barcelona, Spain) were treated with OM-89, an immunotherapeutic product extracted from *Escherichia coli* (OM Laboratories, Geneva, Switzerland), following different administration protocols:

For the monitoring of IgA levels in intestinal secretions, each mouse was treated during 10 days with a total dose of 29 mg of OM-89. Mice were orally given a daily dose of 0.2 ml of the dilution of the drug in saline. Control mice received the same volume of saline.

For the induction of splenic plaque-forming cells (PFC), the mice received different concentrations of OM-89 diluted in 0.5 ml of saline as a single i.p. dose. Control mice received the same volume of saline.

In the experiments on the stimulation of resistance towards *E. coli* experimental infection, the mice were orally treated during 10 days with a total dose of 29 mg of OM-89 as described above. Control animals received the same volume of saline.

In the studies on the modulation of resistance towards *Pseudomonas aeruginosa* infection, mice were treated for 5 days with a total dose of 29 mg of OM-89. Each mouse was orally given a daily dose of 0.2 ml of the dilution of the drug in saline. Control mice received the same amount of saline.

Cyclophosphamide Immunosuppression

Mice were immunosuppressed by a single i.p. inoculation of 200 mg/Kg of Cyclophosphamide (CY, Funk Lab., Barcelona, Spain), diluted in 0.2 ml of saline (7).
Determination of IgA levels in intestinal secretions

Groups of 10 mice for both the control and the OM-89-treated animals were sacrificed by cervical dislocation at days 10, 20 and 25 after the end of the treatment. Each group of 10 mice was processed as a single sample for the determination of IgA levels in gut, as previously described (7,8). Briefly, guts were immediately removed and placed at 4°C. After thoroughly rinsing with saline containing sodium azide (1 g/l), the total content and rinsing solution of the guts were centrifuged at 8,000 x g for 15 min at 4°C. Supernatants were 100-fold concentrated by negative pressure ultrafiltration using 8/32 Visking tubing (Scientific Inst. Ctr. Ltd., London, U.K.) and kept frozen until analyzed. IgA determinations were performed by quantitative radial immunodiffusion (9) using commercially available plates (Meloy Lab. Inc., Springfield, Va.). IgA levels in the concentrated samples were calculated with respect to mg of protein as determined by the Lowry's method (10).

Determination of Direct PFC

Mice were sacrificed 4 days after the i.p. inoculation of OM-89 and had their spleens removed in order to count the number of anti-sheep red blood cells PFC as described by Cunningham (11).

Protection Experiments

In the experiments on the enhancement of resistance towards E. coli, OM-89-treated and control mice received 7 days after the end of the treatment, a single i.p. challenge inoculation of 3.2 x 10⁷ cells of E. coli, belonging to the strains used for the elaboration of OM-89. This bacterial dose approximately corresponds to the minimal LD₁₀₀.

In the experiments on the modulation of resistance to P. aeruginosa, following a modification of the model described by Nugent et al. (12), three different experimental protocols were
used: (i) Mice were orally treated with OM-89 for 5 days starting 3 days after CY-immunosuppression. On the day of the last OM-89 administration, mice were ether-anesthetized and inoculated with 10 ul in each nasal cavity of a \textit{P. aeruginosa} (ATCC 27853) suspension of about $10^{4}$ cells per ml (the minimal i.p. LD$_{100}$ in mice corresponds to $3 \times 10^{4}$ cells/ml). The cell inoculum was given using a Hamilton microsyringe No. 710 (Hamilton Co., Reno, NE.). Mice were kept at low temperature (8-12°C) for the first 15-28 hours after the bacterial inoculation and then placed back at room temperature (22°C).

(ii) As above, but mice were not placed at low temperature after the bacterial inoculation.

(iii) Mice were orally treated with OM-89 for five days; on the day of the last OM-89 administration, mice received the CY and 3 days afterwards were intranasally inoculated with \textit{P. aeruginosa} and kept at low temperature for the first 15-18 hours and then placed back at normal room temperature.

RESULTS

Effect Of OM-89 On IgA Levels In Gut Secretions

The level of IgA in intestinal secretions was determined in groups of 10 mice at days 10, 20 and 25 after the end of oral treatment with OM-89. Each group was processed as a single sample and the results are expressed as the ratio between mg of IgA per mg of protein in OM-89-treated and untreated control mice, in order to avoid individual variability (Fig. 1). The oral administration of OM-89 led to an increase in IgA in gut secretions. IgA in intestinal secretions at days 20 and 25 after the end of the treatment was significantly (p<0.05; Student's t-test) higher in OM-89-treated mice than in controls, reaching for the treated mice twice the levels of that recorded for the controls at day 25. The mean IgA level in control mice was 0.06 mg of IgA per mg of protein.

Enhancement Of Direct PFC Counts

The number of direct splenic PFC in mice was determined 4 days after the i.p. inoculation of OM-89 at different doses. As
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FIG. 1. Effect of the oral administration of OM-89 on the level of IgA in gut secretions. Each value represents the ratio of the mg of IgA per mg of protein in the gut of 10 OM-89-treated and 10 untreated control mice. Results are expressed as the mean of two separate experiments.

shown in Table 1. OM-89 induced a significant (p<0.005, Student's t test) enhancement at doses higher than 0.58 mg, in anti-SRBC PFC, which appear to be dose-dependent (with the highest assayed OM-89 dose being equivalent to the total dose given to mice for the stimulation of IgA in gut secretions), and which induced the highest PFC counts reaching over a 9-fold increase with respect to the control mice.

Protection Experiments

Enhancement of resistance towards E. coli. Mice were challenged 7 days after the end of the OM-89 oral treatment with the i.p. inoculation of E. coli. OM-89-treated mice showed in two separate experiments a mean survival rate of 31.5% (27.5 and 35.5% in the separate experiments) after the inoculation of 3.2 x 10⁶ cells, which corresponds to the minimal LD₁₀₀₀ (Data not shown).

Enhancement of resistance towards P. aeruginosa. Cyclophosphamide-immunosuppressed mice were orally treated with OM-89 for 5 days, starting 3 days after the inoculation of CY. On the last day of the treatment, mice were intranasally challenged with P. aeruginosa and immediately placed at low
TABLE 1

Effect Of The IP Inoculation Of OM-89 In Direct PFC Counts In Spleen.

<table>
<thead>
<tr>
<th>mg of OM-89</th>
<th>No. mice</th>
<th>PFC/spleen</th>
<th>PFC/10⁶ lymph.</th>
<th>I²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>18</td>
<td>9.0</td>
<td>6.2 + 7.3</td>
<td>-</td>
</tr>
<tr>
<td>0.29</td>
<td>6</td>
<td>14.5</td>
<td>9.0 + 9.0</td>
<td>1.4</td>
</tr>
<tr>
<td>0.53</td>
<td>6</td>
<td>45.8</td>
<td>34.0 + 31.1</td>
<td>5.5²</td>
</tr>
<tr>
<td>2.90</td>
<td>12</td>
<td>68.7</td>
<td>47.0 + 29.4</td>
<td>7.6²</td>
</tr>
<tr>
<td>29.00</td>
<td>12</td>
<td>71.8</td>
<td>60.5 + 21.7</td>
<td>9.7²</td>
</tr>
</tbody>
</table>

a: Ratio between the number of PFC per 10⁶ lymphocytes in OM-89 treated and control mice.
b: Significant per p < 0.005, Student's t test.

temperature, where they stayed for the first 15-18 hours after the challenge infection. The results (Fig. 2; Exp. A) show that the oral administration of OM-89 induced in immunosuppressed mice a significant (p<0.05; Student's t test) increase in the protection towards the intranasal infection with P. aeruginosa (mean survival rates of 55 % in OM-89-treated mice and 18 % in control mice). Following the same experimental protocol, one tenth of the dose of OM-89 used in the previous experiment, i.e. a total dose of 2.9 mg, did not induce an enhancement of protection (data not shown). When under the same experimental conditions, mice orally treated with 29 mg of OM-89 were not placed at low temperature right after the inoculation with P. aeruginosa, but kept at normal environmental temperature of the animal facilities, no differences in survival rates were observed between both groups of mice after the challenge infection (Fig. 2; Exp. B). 50 % of the OM-89 treated mice versus 55 % of the control mice survived after the intranasal inoculation of P. aeruginosa. In the third experimental protocol, mice were first given OM-89 orally for 5 days, then immunosuppressed by CY-inoculation the last day of the oral treatment and finally intranasally challenged with P. aeruginosa 3 days later. Under such experimental conditions, no protective
FIG. 2. Resistance to the intranasal infection with P. aeruginosa in CY-immunosuppressed mice, following three experimental protocols: (A) CY-immunosuppressed mice were orally treated with OM-89, intranasally challenged with P. aeruginosa and immediately placed at low temperature. (B) As above, but mice were kept at normal temperature after the bacterial inoculation. (C) Mice orally treated with OM-89 were immunosuppressed by CY-inoculation, then challenged with P. aeruginosa and immediately placed at low temperature. (Results are expressed as the mean of three separate experiments).

effect of OM-89 treatment could be observed (Fig. 2; Exp. C), with mean survival rates of 51% in OM-89-treated mice and 52% in control mice.

DISCUSSION

The mucosal tissues of mammals represent an enormous surface area which includes the intestinal, respiratory and urogenital tracts and the mammary glands. Bienenstock and Befus (13) proposed as a concept that the lymphoid tissue from all these mucosal surfaces constitutes a common mucosal system, in which IgA-precursor cells originating after antigenic stimulation within the mucosal tissue, circulate and lodge mainly in the tissue where they originate from, but also in other mucosae. A number of bacterial agents can affect the host's immunity by modulating its immune responses and/or resistance mechanisms; however few data exist on the effect and mode of action of such immunomodulators when given by the oral
The oral administration of OM-89 to mice induced a significant increase of IgA in gut secretions which was at highest 25 days after the end of the treatment, when a two-fold increase in secretory IgA levels was observed in comparison with the controls.

The OM-89-induced response could be considered either specific or mediated by polyclonal cell activation. In any case, the crucial importance of IgA in mediating the local immune response at a mucosal level (15,16), suggests the potential usefulness of OM-89 in eliciting a prophylactic response against infection. The i.p. administration of OM-89 was able to induce a stimulation of anti-SRBC PFC in the spleen. The effect of OM-89 was dose-dependent and produced in treated mice up to a 9-fold increase in PFC when compared to untreated controls.

The combined effects of both low environmental temperature and CY-immunosuppression enhanced differences in survival rates in protection experiments after the intranasal administration of P. aeruginosa, which in previous experiments by us (unpublished results) and other authors (12), has been shown to produce bacterial lung colonization. In the model of CY-immunosuppressed mice, the oral administration of OM-89 led to a significant increase in the resistance to the intranasal infection with P. aeruginosa when mice were placed at low environmental temperature right after the bacterial challenge. Under the same experimental conditions, no protection could be observed in mice treated with one tenth of the dose administered in the previous experiment.

Similar findings have been reported with other immunomodulators (23), suggesting a dose-dependent effect. No differences in survival rates were observed between OM-89-treated and control mice when they were kept at normal environmental temperature after the intranasal administration of P. aeruginosa. Neither could a protective effect be noticed when the timing of the experimental protocol was inverted, i.e. when mice were immunosuppressed by CY-inoculation after OM-89 treatment, but prior to P. aeruginosa infection. These findings led us to the conclusion that the timing of the different treatments is of crucial importance for the development of protection assays.
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Towards experimental infection. Additionally, the oral administration of OM-89 was found effective in enhancing the resistance to challenge i.p. inoculation with *E. coli*: 31% of the OM-89-treated mice showed resistance to infection with the minimal LD100 seven days after the end of the oral treatment.

Cell-mediated immunity plays also a vital role in local immune defences, although the nature and behaviour of the cells involved remain controversial. OM-89, besides eliciting an immune response, is able to enhance the resistance towards *P. aeruginosa* infection, probably by stimulating cell-mediated immunity as already suggested by Rosenthal (17), and also since the effect can be observed right after the end of OM-treatment. *P. aeruginosa* was selected for the study of the modulation of resistance to bacterial infection, essentially because of the increasing incidence of this opportunistic pathogen for humans in a variety of infections, which number has dramatically augmented over the past decades (18,19). It has been well documented that susceptibility to experimental challenge may be enhanced by factors such as the use of immunosuppressive drugs (12,20,21), or low body temperature (22).

Analysis of polysaccharide composition, performed on OM-89 preparations, revealed the complete absence of ketodeoxyoctanate (KDO; unpublished results). Additionally, for dose ranges < 0.2 mg/Kg, no pyrogenicity caused by OM-89 has been reported (Experimental procedures of OM Laboratories). According to these observations, the possible effect of endotoxin contaminants in the described effects of OM-89 should be discarded.

Although it is difficult to elucidate the exact mechanisms of the effects of OM-89 in mice, not only because of the nature of bacterial products but also because of the complexity of the immune system itself, we can conclude from our studies that OM-89 shows a potential therapeutic value for the enhancement of protection towards infections by opportunistic pathogens, particularly in immunocompromised hosts.

REFERENCES


