Immunostimulation by bacterial components: II. Efficacy studies and meta-analysis of the bacterial extract OM-89

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Abstract

The bacterial extract OM-89 (Uro-Vaxom®) consisting of immunostimulating components derived from 18 Escherichia coli strains is used for the treatment of recurrent urinary tract infections. We investigated in the mouse the immunogenicity of the bacterial extract after oral administration. After repeated administration of OM-89, a specific serum IgG and IgA response against a number of bacterial strains was obtained. Supernatants of cell cultures prepared from the urogenital tract of immunized mice also contained increased levels of strain specific IgG and IgA. We could show a bias towards a Th1 type immune response as indicated by increased IgG2a levels in sera, and increased IFNγ levels in supernatants of spleen cells. These findings may contribute to an understanding of the therapeutic effect of Uro-Vaxom®: the meta-analysis of several clinical studies confirmed that Uro-Vaxom® constitutes an effective prophylaxis for urinary tract infections. © 2000 International Society for Immunopharmacology. Published by Elsevier Science Ltd. All rights reserved.

Keywords: Bacterial extract; Urinary tract infections; Vaccination; Immunostimulation; meta-analysis

1. Introduction

The bacterial extract OM-89, which contains immunostimulatory components extracted from 18 uropathogenic Escherichia coli strains, has been shown to reduce the incidence of recurrent infections of the lower urinary tract in both children and adults [1-7]. Its immunostimulating properties include the stimulation of different immune defense mechanisms: in vitro OM-89 has been reported to enhance the production of tumor necrosis factor α (TNF-α), interleukin-1, interleukin-2, and γ-interferon by human peripheral blood mononuclear cells [8]. We have shown in a mouse model using an in vitro cell culture system,
that OM-89 acts as a polyclonal B-lymphocyte activator in LPS non-responder and responder mice [9,10]. The extract also stimulates macrophages [11,10] and activates natural killer (NK) cells [8,12]. OM-89 administered orally to mice enhances the IgA levels in intestinal secretions [13]. We have demonstrated the in vivo immunogenicity of the extract in mice after parenteral and oral administration [9,14-16]. We could show the reactivity of immune sera against the 18 E. coli strains used for the preparation of the extract, and against defined bacterial cell wall components [17]. In addition, the sera recognized various other pathogenic bacterial strains [16]. In this study we demonstrate, that after oral administration to mice, OM-89 induces increased serum antibody levels against a number of uropathogenic bacterial strains. We also found an increased level of bacterial strain specific IgG and IgA when investigating the antibody production in cell culture supernatants of the urogenital tract. Moreover, we could show a bias towards a Th1 type immune response indicated by increased IgG2a levels in sera and increased IFNγ levels in supernatants of spleen cells. In addition, we show the results of a meta-analysis of several clinical studies confirming that Uro-Vaxom® constitutes an effective prophylaxis for urinary tract infections.

2. Materials and methods

2.1. Immunomodulators and antigens

OM-89 was obtained from OM PHARMA, Meyrin, Switzerland. The lyophilized product contains immunostimulating fractions extracted from 18 E. coli strains. Isolations of outer membranes from two E. coli strains (HK97 and AB2847) were performed as described [18].

2.2. Bacterial strains

The 18 heat killed E. coli strains used for the preparation of the extract OM-89 were obtained from OM PHARMA. Bacterial strains isolated from patients with urinary tract infections (E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus mirabilis, Providencia rettgeri, Enterococcus faecalis) were obtained from A. Serr, Institut für Mikrobiologie und Hygiene, Freiburg, Germany.

2.3. Immunization procedures

Groups of three to five Balb/c mice (female, 6–8 weeks of age) obtained from Max-Planck-Institut für Immunbiologie, Freiburg, Germany were immunized orally by gavage. The extract was dissolved 1 mg/ml in 0.9% NaCl, sera were collected as indicated.

2.4. Determination of serum antibodies

Serum antibody levels were determined by ELISA as previously described [16]. The wells of Elisa plates (Dynex, Denkendorf, Germany) were coated with 10⁶ bacteria/ml of the bacterial strains. Antibodies were detected with peroxidase conjugated rabbit-anti-mouse IgG, rat-anti-mouse IgG1, rat-anti-mouse IgG2a, or goat-anti-mouse IgA (Dianova, Hamburg, Germany; Biozol, Eching, Germany).

2.5. Determination of cytokines in spleen cell supernatants

Mice were sacrificed by cervical dislocation 1 day after the last immunization. Spleens were homogenized using a potter; cell suspensions were washed twice by centrifugation (280 x g, 4°C, 10 min) and resuspended in 20 ml RPMI 1640 containing 5% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. For cytokine determinations, 3 x 10⁶ cells/ml were incubated (200 µl/well) in 96-well-plates (Becton Dickinson, Heidelberg, Germany) in the presence of 50 µl/well Concanavalin A (ConA, 5 µg/ml) for 64 h. Supernatants from the in vitro stimulated cells were applied in dilution rows to Immulon4 HBX-96-well microtiter plates (Dynex), coated with anti-IFNγ (5 µg/ml PBS) or anti-IL4 (2.5 µg/ml PBS) and incubated for 3 h at RT. Biotinylated detection antibodies were diluted (2 µg/ml) in PBS/1% BSA. Assays were performed according to the instructions of the manufacturer (Pharmingen, Hamburg, Germany).
2.6. **Time-resolved fluorimmunoassay (FIA)**

Antibody production of cells of the urogenital tract was determined by FIA as previously described \[15\]. Mice were sacrificed by cervical dislocation 6 days after the last immunization. Tissues were prepared, cut with a scalpel and tissue pieces were incubated with gentle shaking at 37°C for 30 min in 20 ml RPMI 1640 containing 0.75 mg/ml CaCl\(_2\) \(\times 2\)H\(_2\)O, 50 U/ml collagenase type VII (Boehringer Mannheim, Germany) and 150 \(\mu\)g DNAseI (Sigma, Deisenhofen, Germany). The partially digested tissues were homogenized by passing through a syringe, washed twice by centrifugation (280 \(\times\) g, 4°C, 10 min) and resuspended in 20 ml RPMI 1640, containing 5% FCS, 100 U/ml penicillin and 100 \(\mu\)g/ml streptomycin. Microtiter plates (Dynex) were coated with log bacteria/ml. \(3 \times 10^6\) cells/ml, prepared from the urogenital tract of control or immunized BALB/c mice, were applied to the individual wells and incubated for 20 h, 37°C \(\times\) 5% CO\(_2\). As detection antibody biotinylated affinity-purified goat-anti-mouse IgG or goat-anti-mouse IgA (Biozol) was used.

2.7. **SDS-PAGE and Western blot**

Polyacrylamide gel electrophoresis of bacterial outer membrane proteins of *E. coli* strains HK97 and AB2847 was performed according to Laemmli [19] using 10% resolution gels and 5% stacking gels. Gels were silver stained [20] or transferred onto a PVDF transfer membrane (Hybond-P, RPNN303F, Amersham, Braunschweig, Germany) with a Mini-Protean II system (Bio-Rad, München, Germany). PVDF-membranes were blocked for 2 h, 20°C in blocking buffer (20 mM tris(hydroxymethyl)aminomethan/HCl, pH 7.6, 0.5 M NaCl, 0.05% Tween-20, and 5% skimmed milk powder) and then incubated for 20 h at 4°C in serum diluted 1:500 in TBS (20 mM tris(hydroxymethyl)aminomethan/HCl, pH 7.6, 0.5 M NaCl). After washing three times with TTBS (TBS containing 0.05% Tween-20), the membranes were incubated with alkaline phosphatase conjugated goat-anti-mouse IgG (Sigma, Deisenhofen, Germany) diluted 1:1000 in TBS. After washing three times with TTBS and once with TBS, bound antibodies were detected using the NBT/BCIP Western blotting detection system (Boehringer Mannheim, Germany).

2.8. **Statistical analysis**

P-values, Mann–Whitney values (MW) and confidence values were calculated with the program-modul ‘Wilcoxon–Mann–Whitney test’. Test for homogeneity was performed according to DerSimonian [21]. Meta-analysis was performed according to Schemper [22] and Sukhatme [23] using the program StrataPool.

3. **Results and discussion**

In this study we demonstrate that the extract OM-89, prepared from 18 uropathogenic *E. coli* strains, acts as an immunogen in mice after repeated oral immunizations; the data thus confirm and extend our previous investigations \[15,24\]. The antisera obtained recognized *E. coli* strains used for the preparation of the extract and various other uropathogenic bacterial strains [16]. Fig. 1a shows increased bacterial strain specific IgG, and Fig. 1b increased bacterial strain specific IgA against *E. faecalis, P. mirabilis, P. rettgeri, K. pneumoniae, K. oxytoca* and *E. coli* in sera of OM-89 immunized mice in comparison to control sera. As expected, sera of unimmunized mice also recognized the bacterial strains, which may be caused by previous infections, or by the contact of the host immune system with the bacterial population of e.g. the respiratory tract or the gut, or by the presence of polyspecific antibodies [25].

The recognition of different bacterial strains by OM-89 specific antisera might be due to their reactivity with bacterial outer membrane proteins. Fig. 2 shows *E. coli* outer membrane proteins as separated by SDS-PAGE followed by Western blot. Lanes 1 and 2 show the silver stained polyacrylamide gels, lanes 3–8 show Western blots using sera of control mice (lane 3 and 4), orally immunized mice (lane 5 and 6), and intraperitoneally immunized mice (lane 7 and 8). Control sera only weakly recognized the bacterial proteins.
However, sera of orally immunized mice recognized especially proteins with a molecular mass of about 40 kDa; sera of intraperitoneally immunized mice showed the strongest binding to bacterial proteins with a molecular mass of about 90, 40 and 30 kDa. We have already shown in previous studies that the OM-89 specific antisera recognized murein, a common cell wall constituent of Gram-negative and Gram-positive bacteria, and lipoprotein and protein I, common cell wall constituents of Gram-negative bacteria [15].

The increase of the bacteria specific serum IgA after the oral administration of OM-89 indicated a mucosal immune response. To further investigate on the stimulation of the mucosal immune system, we used FIA to determine the specific antibody secretion of B-cells of the urogenital tract and found increased levels of strain specific IgG and IgA (Fig. 3). Similarly, Langermann et al. [26] had shown after immunization of mice with bacterial adhesin or pili the induction of specific IgG against FimH penetrating into the mucosal lining of the bladder and the urinary tract.
Determination of bacterial strain specific serum IgG2a and IgG1 (insert) in OM-89 treated (squares) or control (circles) mice. Groups of three Balb/c mice were immunized orally with 300 μg OM-89 per mouse and immunization in 300 μl 0.9% NaCl on days 1, 14, 29, 43, 57, 105 and bled on days 0 and 119. The control group was not immunized. Pool sera were tested by Elisa for specific IgG1 and IgG2a against the E. coli strains used for the preparation of OM-89. Averages of two-fold determinations ± S.D.

Fig. 4. Determination of bacterial strain specific serum IgG2a and IgG1 (insert) in OM-89 treated (squares) or control (circles) mice. Groups of three Balb/c mice were immunized orally with 300 μg OM-89 per mouse and immunization in 300 μl 0.9% NaCl on days 1, 14, 29, 43, 57, 105 and bled on days 0 and 119. The control group was not immunized. Pool sera were tested by Elisa for specific IgG1 and IgG2a against the E. coli strains used for the preparation of OM-89. Averages of two-fold determinations ± S.D.

tract. The colonization by E. coli of the urinary tract was blocked, and so mice were protected.

To further characterize the humoral immune response we determined OM-89 specific IgG1 and IgG2a in sera of orally immunized mice. Fig. 4 shows that mice immunized with OM-89 predominantly generated antibodies of the IgG2a type. These findings could be supported by the determination of cytokines produced after oral immunization with OM-89. We prepared spleen cells from immunized mice and measured, 1 day after the last immunization, the titers of IFN-γ and IL4 in spleen cell supernatants after in vitro stimulation with Concanavalin A (Fig. 5). The IFN-γ production was clearly increased, both after short time immunization (one immunization cluster, day 1, 3, 5) and after long time immunization (12 immunization clusters). There were no differences in the levels of IL4 production. Our data indicate a bias towards a Th1 type immune response. Th1-dominated responses are highly protective against the majority of microorganisms and usually cause their elimination [27].

Our findings are of importance for understanding the therapeutic effect of OM-89 (Uro-Vaxom®), which constitutes an effective medication in human patients for the prevention of recurrent urinary tract infections, as shown in several clinical trials. We here present a summarizing meta-analysis of results of five double blind, randomized, placebo controlled clinical studies, involving a total of 601 patients. The patients were treated daily for 3 months with one capsule Uro-Vaxom® (6 mg), or with a placebo, and observed for further 3 months without treatment. The efficacy of Uro-Vaxom® was determined with respect to the number of recurrences of urinary tract infections. In Table 1, a summary of the number of recurrences as found in these studies, as well as a statistical evaluation of the results is presented. As seen from the table, the superiority of the Uro-Vaxom group was statistically significant in all studies. Table 2 and Fig. 6 show the results of the meta-analysis of the combined results of the five studies as performed by the program Stratapool. The Mann–Whitney values (MW) of the studies were between 0.621 and 0.755. According to Colditz et al. [28] the following limiting values are of interest for determining the relevance of the superiority of one treatment over the other: 0.5, no difference; 0.56, minor difference; 0.64, medically relevant difference; 0.71, pronounced difference between the two groups. Combining the studies, we obtained a MW of 0.683 (95%: 0.643–0.724); there was no...
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* Test for homogeneity: Chi square = 5.6556; DF = 4 (compare Table 1).
Fig. 6. Results of the meta-analysis according to Mann-Whitney of five clinical trials, as detailed in Table 2.

Indication for heterogeneity ($P = 0.227$, Chi square 5.65, DF = 4). The MW values (fixed effect 0.6834, random effect 0.685) thus demonstrate a statistically significant superiority (lower bound CI: MW > 0.5) and moreover a medically relevant superiority (also lower bound CI: MW > 0.64).

In summary, we could demonstrate in mice that oral immunization with the bacterial extract OM-89 induces increased serum antibody levels against various uropathogenic bacterial strains, and induces a mucosa-associated immune response. Furthermore we could show a bias towards a Th1 type immune response. The efficacy of OM-89 (Uro-Vaxom®) was shown in five different clinical studies and confirmed by meta-analysis: the data demonstrate that Uro-Vaxom® constitutes an effective prophylaxis for recurrent urinary tract infections.

References


