AN Escherichia coli-BASED ORAL VACCINE AGAINST URINARY TRACT INFECTIONS POTENTLY ACTIVATES HUMAN DENDRITIC CELLS

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ABSTRACT

Objectives. To investigate the effects of Uro-Vaxom, an oral vaccine against Escherichia coli urinary tract infections, on human monocyte-derived dendritic cells (moDCs). Dendritic cells (DCs) are important antigen-presenting cells of the immune system. DCs are considered promising cellular adjuvants for inducing immunity against cancer or infectious diseases.

Methods. moDCs were generated in the presence of granulocyte-macrophage colony-stimulating factor and interleukin-4. Flow cytometric phenotyping, as well as the ability to stimulate T cells in an allogeneic mixed leukocyte reaction, was used to assess the effects of Uro-Vaxom on human moDCs. In addition, interferon-gamma and interleukin-4 production by T cells stimulated with Uro-Vaxom–activated moDCs were measured by intracellular fluorescence-activated cell sorter-staining at the single-cell level.

Results. Uro-Vaxom induced the terminal maturation of CD83+ moDCs in a dose-dependent manner. Phenotypic analyses revealed that Uro-Vaxom–activated moDCs displayed a phenotype of mature DCs with high levels of MHC molecules and increased levels of co-stimulatory molecules (CD80, CD86). Consistent with these findings, Uro-Vaxom–activated moDCs potently stimulated T-cell proliferation and interferon-gamma production in the allogeneic mixed leukocyte reaction.

Conclusions. In DC-based immunotherapy, Uro-Vaxom could be used as a stimulant of DC maturation, which meets the standards of good manufacturing practice. In future preclinical studies, we will evaluate the effectiveness of a vaccination with Uro-Vaxom–activated moDCs. It could be an attractive treatment option in preventing recurrent E. coli urinary tract infections in predisposed individuals.


Urinary tract infections (UTIs) are a common cause of morbidity and sometimes mortality. Escherichia coli are the main causative agent of uncomplicated UTIs. E. coli account for more than 85% of cases of acute cystitis and pyelonephritis, as well as for more than 60% of recurrent cystitis and at least 35% of recurrent pyelonephritis.1 Uro-Vaxom was developed as an oral vaccine for patients with recurrent E. coli infections. Orally administered antigen is transported across the epithelium by specialized epithelial cells (M cells) that are located in Peyer’s patches. If transmitted to professional antigen-presenting cells (ie, dendritic cells [DCs] within Peyer’s patches), orally administered antigens can successfully induce specific secretory immunoglobulin A responses.2 IgA is an immunoglobulin that serves to protect the epithelial borders against invading pathogens.3 4 However, administering antigens through the oral route can also induce immunologic tolerance, resulting in a lack of protection. Differences in uptake and handling of orally administered antigens may account for the induction of immunity versus tolerance, yet the cellular or molecular basis remains unclear.

DCs are specialized antigen-presenting cells that are able to initiate immune responses by activating naive and memory T cells. Moreover, they provide...
a key link between the innate and adaptive immune system. In DC differentiation, an immature and a mature stage have been defined: immature DCs are scattered throughout the body where they survey the borders for invading pathogens. Immature DCs pick up antigens by endocytic mechanisms and, after processing, present antigenic peptides on surface major histocompatibility complex (MHC) molecules. Alerted by microbial products such as bacterial lipopolysaccharides (LPS) or viral RNA, they undergo distinct functional and phenotypical changes that have collectively been referred to as maturation. During maturation, DCs stabilize surface expression of MHC-peptide complexes and acquire high-level expression of adhesion and costimulatory molecules, as well as expression of chemokine receptors, which direct DC migration to secondary lymphoid organs. There, antigen-presenting DCs are ideally located to select and activate antigen-reactive CD4+ T helper (Th) cells and CD8+ cytotoxic T cells from the circulating pool of lymphocytes and direct them to the infection site.

Recently, feasible methods to generate DCs from monocytes (moDCs) have been established. In the presence of granulocyte-macrophage colony-stimulating factor and interleukin (IL)-4 peripheral blood monocytes differentiate into immature DCs. Numerous stimuli can induce the irreversible ("terminal") maturation of CD83+, immunostimulatory moDCs. Tumor necrosis factor-alpha (TNF-α) induces the terminal maturation, and prostaglandin E2 can enhance the TNF-α-induced maturation of moDCs. Bacterial LPS, which can induce TNF-α production in various cell types, or double-stranded viral RNA are also effective at promoting the terminal maturation of moDCs. Finally, activated antigen-specific T cells express CD40 ligand and induce moDC maturation by triggering CD40 molecules on the moDC surface.

MoDCs are increasingly used in clinical settings to induce or enhance antitumor immune responses. In these studies, evidence has been obtained that moDCs can indeed be used to immunize patients' antigen specifically and that successful immunization can also be accompanied by clinical responses.

We examined the influence of Uro-Vaxom on human moDCs and show that Uro-Vaxom can potentially activate moDCs that subsequently stimulate T cells with high interferon-gamma (IFN-γ)–producing capacity.

MATERIAL AND METHODS

Uro-Vaxom

Uro-Vaxom (Sanoï-Synthelabo, Berlin, Germany) is a lysozyme composed of immunoreactive fractions of selected E. coli strains that was developed as an oral vaccine against chronic and recurrent UTIs.

CULTURE MEDIUM

RPMI 1640 was supplemented with 10% fetal calf serum, 50 U/mL penicillin, 50 μg/mL streptomycin, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM pyruvate, and 5 × 10−5 M 2-mercaptoethanol.

GENERATION OF moDCs

moDCs were generated from peripheral blood mononuclear cells, as described previously. In brief, peripheral blood mononuclear cells were isolated from heparinized whole blood by standard density gradient centrifugation on Lymphoprep (Axis-Shield, Oslo, Norway). CD14+ monocytes were isolated from peripheral blood mononuclear cells by positive magnetic selection using anti-CD14 antibodies (Miltenyi Biotec, Bergisch Gladbach, Bodenheim, Germany) and a magnetic cell separator, according to the manufacturer's instructions (more than 98% purity). The cells were subsequently cultured in six-well plates (Costar, Germany) at a final concentration of 3 × 106 cells per well in 3-mL culture medium supplemented with 1000 U/mL each of granulocyte-macrophage colony-stimulating factor and IL-4. To induce maturation, moDCs were cultured with different concentrations of Uro-Vaxom (0 to 100 μg/mL), TNF-α (1000 U/mL), or LPS (100 ng/mL) from days 5 to 7.

FLOW CYTOMETRIC ANALYSES

To determine the surface expression of various DC-associated antigens, cell suspensions (106 cells in 50 μL) were labeled for 30 minutes on ice with primary mouse monoclonal antibody in complete medium, followed by fluorescein isothiocyanate-conjugated F(ab')2 fragments of goat anti-mouse Ig (Dako, Glostrup, Denmark). The following monoclonal antibodies were used: TU36 (IgG2b, anti-HLA-DR), HB15A (IgG2b, anti-CD83), BBI (IgM, anti-CD80), and IT2.2 (IgG2b, anti-CD86). The samples were analyzed by flow cytometry (FACScan, CellQuest software from Becton Dickinson, Mountain View, Calif).

PREPARATION OF TH CELLS

CD4+ T cells were positively selected using CD4 microbeads (Miltenyi Biotec) according to the manufacturer's instructions.

MIXED LEUKOCYTE REACTION

CD4+ T cells (2 × 104/well) were stimulated with various numbers of allogeneic, irradiated (3000 rad) moDCs. Cells were cultured in triplicates in 96-well flat-bottomed tissue culture plates in a final volume of 200 μL/well in medium containing 10% fetal calf serum. T-cell proliferation was measured as [3H]thymidine incorporation (μCi/well = 37 kBq/ well; ICN Biomedicals). Cells were pulsed during the last 16 hours of a 5-day culture period, harvested on glass fiber filters using a Skatron cell harvester (Skatron Instruments, Lier, Norway), and analyzed in a liquid scintillation counter. The results are expressed as the mean count per million of triplicate wells ± SD.

T-CELL ASSAYS

moDCs were treated with different concentrations of Uro-Vaxom or LPS at 100 ng/mL. moDCs were cultured with allogeneic CD4+ T cells in a total volume of 200 μL RPMI plus 10% fetal calf serum in flat-bottomed 96-well plates at a 1:300 ratio. From day 2 on, IL-2 (50 U/mL) was added every other
FIGURE 1. Uro-Vaxom induced expression of the maturation-associated CD83 antigen. Day-5 moDCs were treated for 48 hours with Uro-Vaxom at (A) 10 pg/mL, (B) 30 pg/mL, or (C) 100 pg/mL or with (D) TNF-α at 1000 U/mL. CD83 expression was assessed by flow cytometry. Open histograms: IgG2b isotype control.

FIGURE 2. Cell-surface CD83 expression by moDCs. moDCs were cultured with increasing concentrations of Uro-Vaxom (0 to 100 μg/mL) or with TNF-α. CD83 expression expressed in the percentage of expression of the total cell population. One representative experiment of three independent experiments with a similar outcome shown.

day. Intracellular cytokine detection was performed 9 days after stimulation (see below).

CYTOKINE MEASUREMENTS
To detect intracellular cytokines at the single cell level, T cells were stimulated with 10⁻⁷ M phorbol 12-myristate 13-acetate plus 1 μg/mL of ionomycin (Sigma) for 5 hours. Brefeldin A (10 μg/mL, Sigma) was added during the last 3 hours to induce intracellular accumulation of cytokines. Cells were fixed and permeabilized (Fix&Perm, An der Grub, Kaumberg, Austria) and stained with fluorescein isothiocyanate-labeled IFN-γ or phycoerythrin-labeled IL-4-specific monoclonal an-

RESULTS
URO-VAXOM INDUCES MATURATION OF moDCs
To investigate the effects of Uro-Vaxom on the maturation of moDCs, day-5 moDCs were treated with different concentrations of Uro-Vaxom (10 to 100 μg/mL). In control experiments, moDCs were stimulated with TNF-α (1000 U/mL), known for its maturation-inducing capacity, or left unstimulated (medium). Two days later, the phenotype and function of the moDCs were assessed. Phenotypic analyses revealed that Uro-Vaxom at 10 μg/mL had no influence on the maturation of moDCs, and 30 μg/mL led to the expression of the maturation-associated marker CD83 in about 50% of the cells. CD83 expression by most cells was induced with concentrations of 80 to 100 μg/mL (dependent on the individual person tested) (Fig. 1). An increase in the concentration of Uro-Vaxom beyond 100 μg/mL was accompanied by substantial signs of toxicity (data not shown). In addition to a dose-dependent upregulation of the CD83 antigen in moDCs (Fig. 2), Uro-Vaxom also led to a concentration-dependent upregulation of MHC class II molecules and costimulatory molecules (CD80 and CD86) in moDCs. Furthermore, no striking differences were found between the phenotype of moDCs matured with Uro-Vaxom at 100 μg/mL or moDCs matured with 1000 U/mL of TNF-α (Table 1), indicating that Uro-Vaxom could efficiently activate moDCs.
TABLE I. Phenotypic changes of monocyte-derived dendritic cells

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<th>Uro-Vaxom (n)</th>
<th>Control</th>
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![Graph showing T-cell proliferation](image)

FIGURE 3. moDCs stimulated with Uro-Vaxom exhibited increased T-cell stimulatory capacity. Day-5 moDCs were stimulated for 48 hours with Uro-Vaxom at 0, 10, 50, or 100 µg/mL or with TNF-α (1000 U/mL). moDCs were irradiated (3000 rad) and used as stimulators of 2 × 10^5 allogeneic T cells. T-cell proliferation was assessed on day 5 by measuring [³H]thymidine incorporation. Results are expressed as the mean count per million of triplicate wells ± SD. Results are from one experiment, representative of three independent experiments.

**Uro-Vaxom–Activated moDCs Are Potent Stimulators in the Allogeneic Mixed Leukocyte Reaction**

Because mature DCs can activate T cells efficiently, we were interested to assess the T-cell stimulatory ability of moDCs matured in the presence of Uro-Vaxom. It is well established that maturation is associated with an increase in T-cell stimulatory capacity. Figure 3 demonstrates that Uro-Vaxom at 10 µg/mL, which failed to induce maturation, also failed to increase the T-cell stimulatory capacity of moDCs. In contrast, moDCs treated with Uro-Vaxom at 50 µg/mL showed a substantially enhanced ability to induce the proliferation of CD4+ T cells, which was even more pronounced than the CD4+ T-cell proliferation induced by TNF-α-activated moDCs. The strongest proliferation was induced by moDCs stimulated with Uro-Vaxom at 100 µg/mL. (Fig. 3).

**Uro-Vaxom–Activated moDCs Preferentially Induce Th Type 1 Cells for IFN-γ Production**

DCs not only activate Th cells but also influence their differentiation into IFN-γ–producing type 1 (Th1) or IL-4–producing type 2 (Th2) cells. We used intracellular cytokine detection and flow cytometry to investigate the capability of Uro-Vaxom–activated moDCs to induce IFN-γ or IL-4 production in Th cells. moDCs activated with LPS were used as controls. LPS is a major component of the outer cell wall of gram-negative bacteria. LPS, like TNF-α, is a potent stimulant for moDCs and induces TNF-α secretion by moDCs. Figure 4 confirms that moDCs activated with Uro-Vaxom have the ability to preferentially direct the development of Th1 cells producing IFN-γ. In contrast, IL-4–producing Th2 cells were relatively rare among T cells induced by Uro-Vaxom–activated moDCs.

**COMMENT**

The results of this study demonstrate that Uro-Vaxom, an oral vaccine against recurrent UTIs, potently activates human moDCs. Uro-Vaxom promotes the terminal maturation of CD83+ immunostimulatory DCs (Figs. 1 and 2), that subsequently induce the activation and differentiation of IFN-γ–producing CD4+ Th cells (Figs. 3 and 4). IFN-γ–producing CD4+ Th cells are referred to as Th1 cells, which mediate delayed-type hypersensitivity responses. Th1 cells and delayed-type hypersensitivity responses are required to eliminate pathogenic bacteria and viruses.

DCs reside in all body tissues, including the epithelia of the skin, lung, intestine, and urinary tract, the most likely entry sites of pathogens. DCs pick up antigens through fluid-phase and receptor-mediated endocytosis. In addition, DCs respond to contact with pathogens through distinct receptors. A family of so-called toll-like receptors binds LPS or, for instance, double-stranded RNA, and signaling through these receptors induces DC maturation. Activated DCs become migratory and home to adjacent lymph nodes, where they activate T cells specific for bacterial or viral antigens. In addition, DCs support Th cell-dependent B-cell acti-
Uro-Vaxom-activated moDCs were compared with LPS-activated moDCs in the stimulation of cytokine release by allogeneic CD4+ T cells. After 9 days, IFN-γ and IL-4 were determined by intracellular fluorescence-activated cell sorter staining after stimulation with phorbol 12-myristate 13-acetate and ionomycin. Results expressed as dot blot (IFN-γ on the x-axis, IL-4 on the y-axis) of the total cell population ganged in the fluorescence-activated cell sorter. One representative experiment of three independent experiments with a similar outcome is shown.

moDCs are frequently used in clinical cancer trials to induce or enhance tumor immunity.15,17,23 We have used CD83+, immunostimulatory moDCs loaded with tumor lysate, and the control antigen keyhole limpet hemocyanin to treat 35 patients with metastatic renal cell carcinoma. This treatment modality was very well tolerated and induced clinical responses in up to one third of the patients17,18 (Holtl et al., unpublished results). Importantly, DC-based immunization induced measurable responses against the control antigen keyhole limpet hemocyanin in all patients tested, indicating that moDCs are promising adjuvants for antigen-specific vaccination of patients. The immune response to entry of pathogens into the urinary tract has been shown to be local and systemic.22 The data presented here suggest that moDCs loaded and activated with Uro-Vaxom may be used as a vaccine for recurrent UTIs. Uro-Vaxom-pulsed moDCs may be injected intradermally close to or into the lymph nodes that drain the urinary tract. Intradermal injection of Uro-Vaxom-pulsed DCs instead of the oral administration of Uro-Vaxom alone may reduce the risk of inducing tolerance. With additional preclinical data, a rationale will exist for further evaluating the safety and effectiveness of Uro-Vaxom in clinical trials for patients with recurrent UTIs. The use of bacterial cultures established from individual patients with recurrent UTIs instead of Uro-Vaxom may further enhance the mucosal immunity of the urinary tract and thus the therapeutic benefit of such a treatment modality.

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REFERENCES


