Eradication of multi-resistant *Escherichia coli* from a patient with urinary tract infection using specific autovaccination therapy

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Nolte, O., Bindewald, A., Weiss, H.-E., Weiss, H., & Sonntag, H.-G.

Hygiene Institute, Dept. of Hygiene & Medical Microbiology, Im Neuenheimer Feld 324, D-69120 Heidelberg
Tel.: +49/6221/567812; Fax.: +49/6221/565627; E-mail: Oliver_Nolte@med.uni-heidelberg.de

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AUTOVACCINES are therapeutic vaccines prepared from a micro-organism isolated from a site of infection. Autovaccines are produced for that particular patient from whom the strain was isolated. They are therefore patient- and strain-specific therapeutics intended to stimulate an immune response against the disease causing micro-organism. Hitherto, little is known about the activation of effector mechanisms after an autovaccine being administered to a patient in case of chronic microbial infection. We wanted to focus on two questions: (i) is autovaccination a useful method for treatment of urinary tract infection (UTI) as described previously [1], and (ii) which effector mechanisms are activated after administration?

Background:

* male patient with UTI over a period of more than six weeks
* unsuccessful treatment with antimicrobial drugs
* microbial content >10^6 *E. coli* (resistant to 4 antimicrobial drugs)/ml urine

According to the procedure outlined in ***Fig. 1*** the autovaccine was manufactured by using the isolated *E. coli* strain.
Autovaccination and sampling of blood and urine followed the schedule given in ***Fig. 2***. Experimental procedure is given in ***Fig. 3***.

Methods

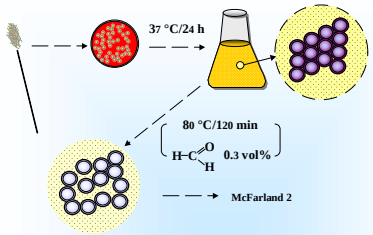


Fig. 1: Manufacturing of autovaccines (see *Ref.* [2])

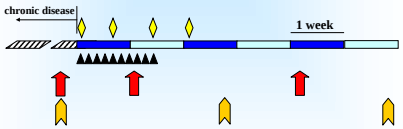


Fig. 2: Diagrammatic representation of disease state, immunisation schedule and sampling of blood. (▲) denotes oral administration, (◊) denotes subcutaneous immunisation, (▯) denotes blood and (▯) denotes urine sample

Results:

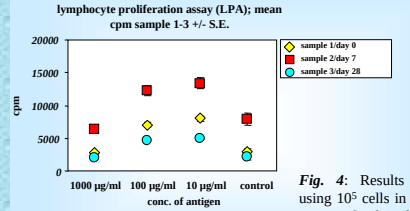
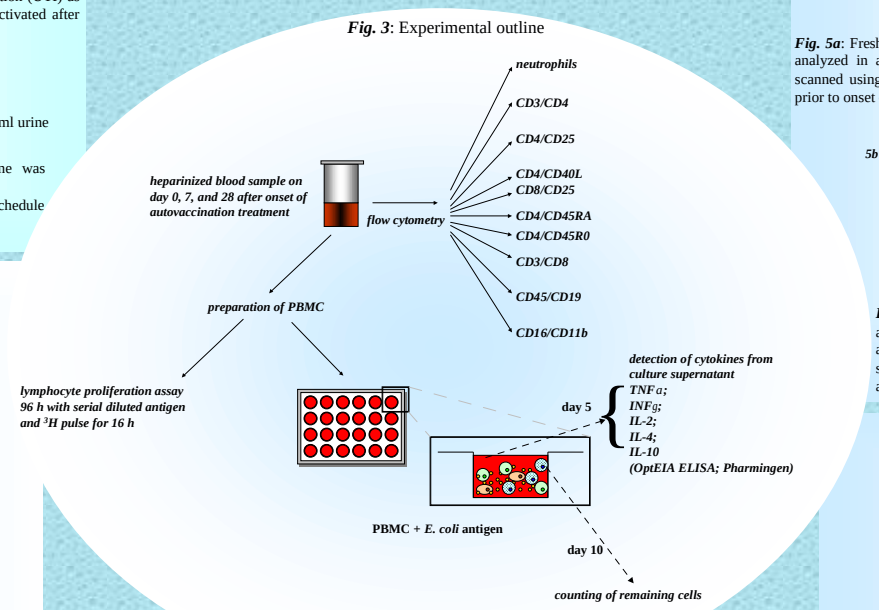


Fig. 4: Results of LPA using 10^5 cells in 6 parallel assays. The data show the highest mean proliferative response of PBMC obtained on day 7 after onset of autovaccination. The response decreased towards the 3rd sample which was obtained on day 28 after start of vaccination therapy.

Fig. 3: Experimental outline



20 days after start of autovaccination therapy *cfu* in urine had declined from >10^6 to <10^4, 35 days (5 weeks) after start of therapeutic vaccination, *cfu* and laboratory findings were within normal range!

Conclusion:

Specific therapeutic autovaccination seems to be a useful alternative for the treatment of chronic UTI.
Autovaccination may lead not only to eradication of a pathogenic micro-organism but also to a (positive) modulation of the patients immune system. However, a scientific proof for the actual usefulness as well as for the mechanism of action is still lacking, although we present here first detailed analysis of changes in immunological parameters which are concerned with the application of an autovaccine.

Our data suggest that soon after start of treatment effector mechanisms of the innate immune system are activated. In particular, we have observed a pronounced dynamic of neutrophils (***Fig. 5a***) in peripheral blood samples, indicating a possible role of these cells in recovery from UTI. This is in good accordance with results observed in a mouse model of urinary tract infection [3].
Short term cultures of lymphocytes in the presence of antigen revealed a peak secretion of INFγ in cultures using cells from day 7 after start of autovaccination whereas the secretion of TNFα increased over the three sampling dates. Classical T_H1/T_H2 cytokines (IL-2 and IL-4) were not detected (***Fig. 6***). Also exogenous rhIL-2 did not increase antigen specific cell proliferation (***Fig. 7***) over the three sampling dates, supporting the finding of activation of innate effector mechanisms in response to the application of the autovaccine.
Antigen specific proliferation of PBMC (***Fig. 4***) revealed the highest responsiveness for the sample obtained on day 7 whereas the responsiveness, however, was lower on day 28 post-vaccination.

References: (1) Allen, R.W. (1914): Die Vakzintherapie. Ihre Theorie und praktische Anwendung, Verlag Steinkoppf, Dresden. (2) Weiss, H.-E., Bertl, F., & Geßler, K. (1998): Heilerfolge durch therapeutischen Einsatz von Pseudomonas aeruginosa Autovakzinen in der HNO-Infektionskasuistik der Kleintierpraxis. Eine Alternative auch für den Menschen? Tierärztl. Umschau 53:38-43. (3) Haraoka, M., Hang, L., Frendéus, B., et al (1999): Neutrophil recruitment and resistance to urinary tract infections. JID 180:1220-119

flow cytometry of whole blood samples

5a

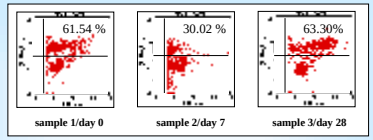


Fig. 5a: Freshly drawn (<4h) heparinized whole blood was lysed and subsequently analyzed in a FACScan (Becton Dickinson). Samples (*5000* lymphocytes) were scanned using identical instrument settings. Day 0 represents the cell distribution prior to onset of autovaccination therapy.

5b

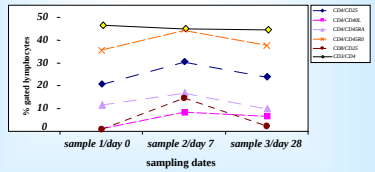


Fig. 5b: Surface markers which were observed to change in percentage are given. Those markers which did not show changes throughout the autovaccination process were omitted from that presentation. It can be seen that cells expressing certain activation markers (CD40L/CD25) are increased on day 7 after start of autovaccination

cytokine secretion in the presence of 0.1 mg antigen per 10^6 cells

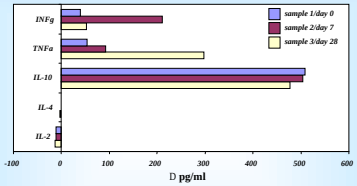


Fig. 6: Determination of various cytokines in supernatants of 5 day short term cultures in the presence of 0.1 mg of heat-killed *E. coli* as antigen. The amount of cytokine given represents the net production in response to the antigen calculated as

D pg/ml = pg per ml cytokine (antigen)/pg per ml cytokine (control).

determination of viable cells after 10 day incubation with 0.1 mg antigen per ml

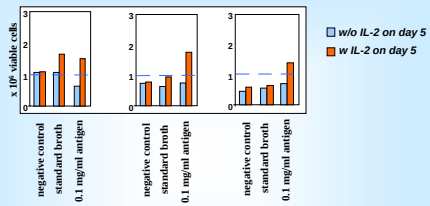


Fig. 7: 10^6 PBMC were cultured in the presence of 0.1 mg/ml heat-killed *E. coli* for 10 days. The remaining viable cells were then counted in a Neubauer chamber. On day 5 supernatants were saved for analysis of cytokine secretion (see ***Fig. 6***) and one of each duplicate culture was stimulated using 20 I.U. of recombinant human IL-2 (R&D Systems). Results of cell counts are given as the mean of two duplicates. The results indicate that the addition of rhIL-2 had no effect on cell proliferation.