Study cells immune response by using a killed suspension of *Pseudomonas aeruginosa* as oral vaccines.

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Abstract

This study was conducted in order to investigate the effect of *Pseudomonas aeruginosa* vaccine on stimulating of cell mediated immune response in laboratory white mice. The result revealed the high increased weight of spleen and mesenterial lymph nodes in the vaccinated animals in contrast to the organs of the control group. The morphological changes in these organs indicate indirectly the high immunological activities of them, beside the activation of kupffer’s cells in the liver of the vaccinated lab. animals.

This study appeared a high interaction between the cellular immune system and the orally administrated whole cell of *Ps. aeruginosa* bacteria.

**Key words:** Ps. aeruginosa, immune response, White mice
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**Introduction**

*Pseudomonas aeruginosa* is the predominant pathogen in patients with cystic fibrosis (CF). Many study showed that none of the vaccines could completely prevent chronic liver inflammation 4 weeks after challenge. However, the changed pathologic condition in immunized mices to a chronic-type inflammation might be of great benefit in future management of CF patients since the developing liver tissue damage has been shown to be caused by polymorphonuclear leukocyte-released elastase[1]. Cystic fibrosis is a genetic disease in which persistent respiratory infection, usually due to *P. aeruginosa* infection[2]. Experiments using absorbed sera showed that the protective antibodies are specific to outer membrane proteins. Thus, live-attenuated *P. aeruginosa* vaccines delivered nasally protect against corneal infections in mice and potentially can be used to prepare passive therapy reagents for the treatment of established *P. aeruginosa* corneal infections caused by diverse LPS serogroups [3]. Patients with cystic fibrosis and with no prior history of infection with *Pseudomonas aeruginosa* were immunized with an octavalent O-polysaccharide-toxin A conjugate vaccine. During the next 4 years, 16 patients (61.5%) remained free of infection and 10 (38.5%) became infected. Total serum anti-lipopolysaccharide (LPS) antibody levels induced by immunization were comparable in infected and noninfected patients[4]. Immunization evoked a vigorous IgA1 and IgA2 antibody response to LPS, which was long-lived. Only a modest, transient IgA antitoxin A response was noted. Both antitoxin A--neutralizing and opsonic antibodies were elicited by immunization and remained elevated over the 14-month postimmunization period studied [5]. *Pseudomonas* exotoxin A has been shown previously to induce suppression of the murine immune response. The addition of 10(-4) ng of exotoxin A induced suppression of the immune response to trinitrophenylated Ficoll from days 3 to 10, while 10 ng of toxin exerted no suppressive effect over the same examination periods [6].

The high levels of antibiotic resistance that occur in *Pseudomonas aeruginosa* have led to growing interest in the development of pseudomonas vaccines. The polyvalentantigen mixture was effective as a vaccine in burned patients. Vaccination produced IgG that was highly specific for the corresponding Immunotype[7].
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*Pseudomonas aeruginosa* is the major infectious agent of concern for cystic fibrosis patients. Strategies to prevent colonization by this bacterium and/or neutralize its virulence factors are clearly needed. Here we characterize a dual-function vaccine designed to generate antibodies to reduce bacterial adherence and to neutralize the cytotoxic activity of exotoxin A., and, when injected produced antibodies that reduced bacterial adherence and neutralized the cell-killing activity of exotoxin A. *Pseudomonas* exotoxin A (here called PE), a prominent virulence factor secreted by *P. aeruginosa*, is cytotoxic for mammalian cells [8]. Examination of lung, liver and spleen tissues isolated 4, 8 and 12 h following intratracheal instillation with PE demonstrated specific cell damage in these tissues which was not observed in mice dosed with ntPE [9].

**Materials and Methods**

This study included (10) white mice (C. F. L. P) and (2) white mice as a control group were used in this study. The weight of the mice is (25) gm. *Pseudomonas aeruginosa* used as a bacterial vaccine in this study after killed by heating, then equal doses of vaccine were prepared and concentrated to $2 \times 10^8$ bacterial cell per milliliter and injected the mice. Then after (4) weeks of the last dose of the vaccine, do tissue sections for all vaccinated and control mice with manual procedure by killed the mice and take the tissues like liver, spleen, and lymph node from it, then put them in the formalin 10% for fixation, then washing it, then dehydration by using 70% alcohol for 30 min, then clearing by using acetone in 4 changes for 80 min. (20 min. for each change) after this clear the tissues by xylene for 30 min., then put them in melting wax for 3 hours 9 infiltration) then we do the blocking (embedding) tissues in paraffin wax by using L-shaped moulds, then do trimming after it do sectioning by using the microtome, then do the mounting by using the water bath, in the last do the staining[10].

**Results**

Table 1 shows the weight of spleen and mesenterical lymph nodes (MLN) of the vaccinated and control groups of the animals used in this experiment.
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Table 1: The weight (gm) of the spleen and mesenterical lymph nodes (MLN) for the vaccinated and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen wt. (gm)</th>
<th>MLN wt. (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated</td>
<td>0.307 ± 5</td>
<td>0.215 ± 5</td>
</tr>
<tr>
<td>Control</td>
<td>0.160 ± 2</td>
<td>0.048 ± 4</td>
</tr>
<tr>
<td>Difference</td>
<td>0.147</td>
<td>0.167</td>
</tr>
</tbody>
</table>

Table 2 shows the histological changes of the spleen, MLN and liver in the vaccinated animals.

Table 2: The histological changes on the white mice spleen, MLN, small intestine, and liver

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histological changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>Prominent white pulps and prominent extramedullary haematopoesis including megakaryocytes</td>
</tr>
<tr>
<td>MLN</td>
<td>Lymphoid hyperplasia of the mesenterical lumph nodes with megakaryocytes</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Show mild to moderate infiltration of the mucosa by plasma cells and lymphocytes and occasional prominent lymphoid aggregate (payer’s patch)</td>
</tr>
<tr>
<td>Liver</td>
<td>Activation of Kupffer’s cells</td>
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</tbody>
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Figure 1 shows mild to moderate infiltration of the mucosa by plasma cells and lymphocytes and occasional prominent lymphoid aggregate, and figure 2 shows small intestinal mucosa with prominent lymphoid hyperplasia from lab. After given the Ps ae. Vaccine, while figure 3(A,B) shows the diffuse lymphoid hyperplasia of lymph nodes, and figure 4 shows a section of liver of the vaccinated mice given oral Ps. ae. Vaccine with activation of Kupffer’s cells.
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*Figure 1:* Small intestinal mucosa of control groups.

*Figure 2:* Small intestinal mucosa with prominent lymphoid hyperplasia from Lab. Animals (Mice) given the Ps. ae. Oral vaccine.
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**Figure 3-A:** Diffuse lymphoid hyperplasia of lymphnode.

**Figure 3-B:** Diffuse lymphoid hyperplasia of lymphnode.
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**Discussion**

Most serological studies of infections with *Ps. aeruginosa* have concentrated on circulating antibodies, but there is also evidence to suggest that local or secretory antibody may have an important role in host defense mechanisms.

The highly increased weight (about double weight) of spleen and MLN in the vaccinated animals (Mice) as shown in table 1, in contrast to the organ of the control treatment animals group. Also the morphological changes in the spleen and MLN as shown in table 2 and figures (1, 2, 3, 4), indicate indirectly the high immunological activities of these organs, beside the the activation of kuffer’s cells in the liver of the vaccinated lab. Animals. The difficulties, which we met in measuring the T-lymphocytes in lab. Animals, enforced us to choose this indirect simple method. One of the major aspects of lymph nodes enlargement is the nodes were filtered and mostly seen in infections. These results are in agreement with Jennifer et al.[11], who found that Vaccination of the mice prior to treatment resulted in better survival and lower bacterial loads compared to vector-immunized mice. Although the treatments had no effect on antibody titers, this level of protection was still lower than that
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seen in untreated vaccinated mice. Administration of antibodies directly to the site of infection at the time of bacterial delivery prolonged survival and lowered bacterial loads in the immunocompromised mice. These results demonstrate the importance of white blood cells while still suggesting a critical role for antibodies in protection against *P. aeruginosa* infection. The lymph nodes serve as the primary site of local defense against antigens, and the spleen as the principal systematic defense. The oral immunization methods offer many advantages over the parenteral techniques that are currently available. The basis question is whether oral prophylaxis is likely to be effective in achieving the desired level of immunity. This question is positively support based on the increasing weight and the morphological changes in the histology of the spleen and MLN, which achieved in the vaccinated lab. Animals, that assure the presence of an interaction between cellular immune system of the lab. Mice and orally administered whole cell of *Ps. aeruginosa* bacteria. Also these results are in harmony with Stanislawsky et al.[12], who found that *Pseudomonas aeruginosa* vaccine (PV) containing cell proteins with molecular weight ($M_r$) 20 000–100 000 and up to 0.08% (M/V) admixture of lipopolysaccharide was obtained by water—salt extraction and subsequent ultrafiltration. PV protects mice against experimental *P. aeruginosa* infection, stimulates production of specific protective antibodies in rabbit and does not provoke obvious toxicity in laboratory animals.

**References**


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