INFLUENCE OF HUMAN RECOMBINANT INTERLEUKIN-1BETA ON PROTECTIVE AND IMMUNOGENIC EFFICACY OF LIVE PLAGUE VACCINE

Vaccination against plague is an important element of control over this exceptionally virulent infection. To be effective against virulent Yersinia pestis strains live plague vaccine produced from Yersinia pestis EV strain (EV vaccine) requires annual revaccinations. Use of EV vaccine for revaccination is limited due to the initially developed immune response that suppresses the live vaccine culture. Earlier studies showed that the use of immunomodulators activates immune response to vaccination.

In our study we assessed the influence of human recombinant interleukin-1beta (Betaleukin) on immunogenic and protective efficacy of live plague vaccine in controlled experiments on animal models (rabbits and guinea pigs). In a long experiment (261 days) on rabbits we assessed indicators of antigen specific immune response to F1 antigen of Yersinia pestis. The early antigen specific response was evaluated based on the appearance of different avidity lymphocytes with F1 receptors. Effector phase of the immune response was assess based on the activity of antigen-specific antibodies.

Results showed that the use of betaleukin as an immunoadjuvant increases vaccination efficacy by strengthening the effector phase of the immune response and promotion of the early stage of antigen-specific immune response to EV vaccine. Protective efficacy of betaleukin and EV vaccine combination was assessed in an experiment with guinea pigs. This experiment showed that injections of betaleukin facilitates the production of antibodies following vaccination and significantly increases the rates of survival after the challenge with virulent plague strain. At the same time single injections of betaleukin alone did not protect guinea pigs from death after injections of virulent plague strains.

Keywords: Human recombinant interleukin-1beta, betaleukin, live plague vaccine, F1antigen, lymphocytes with F1-receptors, antibodies


Introduction

Plague is an exceptionally virulent infection that is still a subject to the International Health Regulations and notifiable to the World Health Organization. It is caused by the Yersinia pestis bacteria that circulates in animal reservoirs, particularly in rodents, in the natural foci of infection found on all continents except Australia (Dennis et al., 1999). Plague bacteria are most often transmitted by the bite of an infected flea or while buchiring an infected animal (Rall, 1965; Domoradskyi, 1998). In Kazakhstan the natural foci of plague cover approximately 40% of the territory of Kazakhstan (Atshabar et al., 2012). Humans living and working in natural foci are extremely susceptible to plague and may be infected. Cumulatively there were 27 cases of human plague registered in Kazakhstan since 1989 with the last case registered in 2003. All registered infection occurred following hunting and butchering wild rodents (marmots) or a sick camel (Bekshin et al., 2012).
Presence of natural foci requires continuous control over the potential spread of infection. Vaccination of people who live or work in natural plague foci is an important element of epidemiological surveillance and infection prevention and control (Feodoroval and Motin, 2012). Vaccination also plays an important role in the efforts to mitigate consequences from potential acts of biological terror (Inglesby et al., 2000).

A live plague vaccine produced from Y. pestis EV strain (EV vaccine) that is widely used in Russia and Kazakhstan decreases the incidence of plague by an average of 10 times and prevents mortality. Use of this vaccine requires annual revaccinations. While being effective during the initial vaccination, effectiveness of live EV vaccine is, however, limited during revaccinations due to the initially developed immune response that suppresses the live culture from which the vaccine is produced. The immunity level after the first vaccination is however not sufficient to protect against virulent Y. pestis strains (Feodoroval and Motin, 2012). Use of various immunomodulators can activate the immune response to vaccination. A synthetic product Polyoxygenium developed by the Institute of Immunology in Moscow is one of the potential immunomodulators (Petrov and Khaitov, 2010). Earlier studies conducted by our group on guinea pigs showed that Polyoxygenium developed by the Institute of Immunology in Moscow has significant immunomodulating (adjuvant) effect when applied together with EV vaccine. A number of infectious models, including several using herpetic vaccines, showed immunomodulating effect of interleukin-1 (IL-1) (Ponomaryova et al., 2010; Deryabin et al., 2012). Interleukin 1 beta (IL1B), a cytokine that participates in inflammatory reactions was first described in 1985 (March et al., 1985). Currently this cytokines that are widely used to stimulate immune response, specifically to stimulate neutrophil and lymphocytes, during the treatment of different infections are also used to improve vaccination schemes (Medunitsin, 2010). However, the use of IL1B to modulate the immune response during vaccinations of animals and humans remains limited (Simbirtsev et al., 2010; Omarova et al., 2011; Karalnik and Denisova, 2011). At the same time, it is the presence of the component that can stimulate synthesis and secretion of cytokines, that can determine effectiveness of vaccines (Petrov and Khaitov, 2010). Results of a control trial on animal models and clinical trials on patients with an onset of herpes showed that proinflammation cytokine administered during immunization with herpetic vaccine stimulates an early antigen-specific response, i.e. development and disappearance of herpes antigen specific lymphocytes, and an effector phase of antigen specific response, i.e. appearance of antibodies (Omarova et al., 2011; Karalnik and Denisova, 2011).

Study design and objective

This was experimental study consisting of two randomized controlled trials using two types of animal models (rabbits and guinea pigs) aimed at assessing the influence of human recombinant interleukin-1beta (beta-leukin) on immunogenic and protective efficacy of live plague vaccine in animal models.

Materials and methods

The study protocol was approved by the institutional review board on bioethics of Aikimbaev Kazakh Scientific Center for Quarantine and Zoonotic Infections, Almaty, Kazakhstan (protocols number 3-8 from). As mentioned above the study involved two animal models. Rabbits (8 animals, 2-3 kg each) that allow frequent blood collections were used to assess antigen-specific response to EV vaccination. Guinea pigs (115 animals, 250-300 gr each) that are historically used as animal models to assess protective effectiveness of EV vaccine since 1936 (12) were used to assess immunogenic and protective effectiveness of the vaccine.

Intervention included injection of beta-leukin produced by LLP “Cytokine”, Russia, and a live Y. pestis EV vaccine produced by the Aikimbaev Kazakh Scientific Center for Quarantine and Zoonotic Infections, Kazakhstan. Activity of antibodies to F1 was
assessed using reagent consisting of antigen-sensitized erythrocytes also produced by the Aikimbaev Kazakh Scientific Center for Quarantine and Zoonotic Infections, Kazakhstan.

The influence of immunomodulating product (betaleukin) on immunogenic efficacy (antigen specific immune response) of EV vaccine was assessed in an experiment on two groups of rabbits (4 animals weighting 2-3 kg in each group) lasting for 231 days. All animals in both control and experiment group received intravenous injections of EV vaccine (3·10^8 cells) diluted in 0.5 ml of 0.85% Sodium chloride solution. Animals in experiment group also received a single injection of 0.5 mcg betaleukin diluted in 0.5 ml of 0.85% Sodium chloride solution. Controls received placebo (single injection of 0.5 ml of 0.85% Sodium chloride solution). Blood from the marginal ear vein was collected from rabbits before the injections and after 2, 4, 7, 14, 21, 28, 35 and 42 days following the injections; to isolate lymphocytes ~4 ml of blood was collected using heparinized blood collection tubes, and ~1 ml of blood was collected to produce serum using tubes without any additives. After 42 days following the injections and until 231 days following the injections blood was collected once a week using tubes without any additives.

During the second stage of our study we assessed the influence of betaleukin on immunogenic (antigen specific) and protective efficacy of EV vaccine in an experiment on guinea pigs (one experiment and two control groups, each animal weighted 250-300 gr). Animals from the experiment group (23 guinea pigs) received intravenous injections of Y. pestis EV vaccine (10^8 microbial cells) and 0.5 mg of betaleukin simultaneously. Controls from group 1 (82 guinea pigs) were injected with Y. pestis EV vaccine (10^6 microbial cells) only. Controls from group 2 (10 guinea pigs) we injected with 0.5 mcg of betaleukin only. 21 days after the injections all animals were challenged by inoculation of Y. pestis virulent 231 strain (200 Dcl). Samples of blood were taken from animals’ hearts, serum was received using the standard method. Animals that remained alive following the challenge were sacrificed by exsanguination with prior application of ether.

**Measurable outcomes**

Immunogenic efficacy was assessed by the titre of antibodies to capsule antigen F1 (activity of antibodies to F1 antigen), as well as the death rate among animals following 14 days after the exposure to Y. pestis virulent 231 strain and the presence of Y. pestis 231 strain in animal spleens. To assess antigen-specific response following the vaccination we measured the level of lymphocytes with receptors to F1 antigen of Y. pestis (LR-F1) and titre of antibodies to capsule antigen F1.

**Data analysis and interpretation**

Statistical analysis was performed and results were considered statistically significant if P≤0.05. The following laboratory methods were used to measure key study outcomes:

1. **Presence and level of LR-F1**

Isolation of lymphocytes was performed in a density gradient (ficoll - verografin) of 1.077 g/cm^3. Presence of LR-F1 was assessed using binding of optimal and suboptimal immunoreagents with isolated lymphocytes at the following proportion: 1:2·10^8 corpuscles of reagent to 2·10^6 lymphocytes (13). In controls, erythrocytes not sensitized to F1 were used instead F1-specific immunoreagents. Presence of LR-F1 and lymphocytes that bind with control reagents in suspension of lymphocytes was counted in percentages. In every batch the quantity of LR-F1 and lymphocytes that bind with control reagents was counted independently in seven hundreds of lymphocytes. LR-F1 counted positively if the quantity of LR-F1 that bind with control reagent was significantly higher (P≤0.05) than the quantity of lymphocytes that bind with control reagent. Special immunoreagents were developed to detect LR-F1 specific to F1. F1 antigen was obtained using method described by Baker (1952). Bovine erythrocytes fixed by acetaldehyde (CH₃-CHO) were
used as a sorbent to fix F1 (Karalnik and Leschinskaya, 1981). Aqueous rivanol solution was used to sensitize erythrocytes with IgG (Shamardin and Karalnik, 1981). To identify F1 doses sufficient to ensure optimal and suboptimal sensitivity of immunoreagents erythrocytes were sensitized by F1 solutions in dilutions from 3.9 to 1000.0 mcg/ml.

**Figure 1. Sensitivity of immunoreagents at different doses of F1-specific antigen**

Produced experimental series of immunoreagents were tested on lymphocytes of rabbits immunized with EV vaccine 15 days before the experiment. Optimal and suboptimal (50%) sensitivity of reagents during LR-F1 isolation was reached at concentrations of 125 mcg/ml and 25 mcg/ml respectively (Figure 1). Total LR-F1 was assessed using the optimal reagent, high-avidity F1-specific lymphocytes was assessed using suboptimal concentrations of LR-F1.

2. Presence and activity of antibodies to F1

Activity of antibodies to F1 was assessed by indirect haemoagglutination test (IHA) using reagent consisting of antigen-sensitized erythrocytes. Antibody response in serum was presented as Ig IHA titre. IHA was performed using polystyrene microtiter plates (17). Each plasma sample was tested four times. The same series of reagent was used during the study.

3. Presence of Y. pestis 231 strain

Bacteriological investigation of spleens from died and sacrificed animals was done using spleen cells plated on Hottinger agar (pH 7.1 ± 0.1).

**Results**

During the first stage of the study we assessed the dynamics of antigen specific immune reaction of rabbits immunized with live EV vaccine with and without the use of betaleukin. Dynamics of LR-F1 isolation with different immunization schemes is presented in Figure 2-3. As shown, level of high-avidity LR-F1 in both schemes is significantly lower than the level of total LR-F1.

As seen from Table 1 betaleukin can significantly (P <0.01) promote the development of the early stage of antigen-specific response (LR-F1) both in terms of the total LR-F1 count and the high-avidity LR-F1 count.

Antibody reaction during the experiment (231 days) is showed in Table 2. Antibodies in a titre ≥1/1000 were detected 28 days following immunization without immunomodulator
and 21 days following immunization with betaleukin. Mean antibody reaction (lg IHA titre) was higher with betaleukin.

**FIGURE 2. LEVEL OF LR-F1 FOLLOWING IMMUNIZATION WITH BETALEUKIN**

![Graph of LR-F1 levels following immunization with betaleukin](image1)

**FIGURE 3. LEVEL OF LR-F1 FOLLOWING IMMUNIZATION WITHOUT BETALEUKIN**

![Graph of LR-F1 levels following immunization without betaleukin](image2)

**TABLE 1. EFFECT OF BETALEUKIN ON LR-F1 LEVELS FOLLOWING EV VACCINATION**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Results following vaccination without immunomodulation</th>
<th>Results following vaccination with betaleukin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of days needed to reach the maximum total LR-F1 count</td>
<td>28</td>
<td>4-7</td>
</tr>
<tr>
<td>Maximum total LR-F1 count, %</td>
<td>12.1±0.14</td>
<td>5.9±0.14</td>
</tr>
<tr>
<td>Last day when total LR-F1 were detected</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Cumulative LR-F1 count, %</td>
<td>266.9</td>
<td>47.0</td>
</tr>
<tr>
<td>Number of days needed to reach the maximum high-avidity LR-F1 count</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Maximum high-avidity LR-F1 count, %</td>
<td>9.93±0.14</td>
<td>3.25±0.10</td>
</tr>
<tr>
<td>Last day when high-avidity LR-F1 were detected</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Cumulative high-avidity LR-F1 count, %</td>
<td>205.6</td>
<td>16.2</td>
</tr>
</tbody>
</table>
Maximum antibody response was recorded 56 days after the vaccinations followed by betaleukin and 119 days following vaccinations without immunomodulation. The duration of immune response following injection of betaleukin was much longer (63 days), compared to only one day of the maximum immune response following vaccinations without immunomodulator. At the end of the experiment (33 weeks after the immunization) mean antibody response (lg IHA titre) was higher when using betaleukin (p <0.001). The total antibody immune response was also higher when using betaleukin.

Results of guinea pigs immunizations with EV vaccine are shown in Table 3 below. Antibodies titre was recorded in 3 guinea pigs from the experiment group and 8 from the control group. There were no deaths among animals during the period between the collection of blood (14 days after vaccination) and their infection (on the 21st day). Antibodies titre was significantly higher (P<0.05) in animals from the experiment group (1:1400) compared to the control group (1:800).

In the control group 1 52 animals died after they were challenged with the injection of Y. pestis virulent 231 strain (200 Dcl). Surviving animals were sacrificed on the 14th day following the challenge. Y. pestis 231 strain was isolated from the spleens of all guinea pigs that died after the challenge and 10 out of 30 surviving animals (33.3±8.6 %). In the experiment group only 4 animals died 14 days after the challenge, which is significantly lower than in the control group (P<0.01). All surviving animals in the experiment group...
were sacrificed 14 days after the challenge. Neither died nor sacrificed animals in the experiment groups had Y. pestis strain in their spleens, which proves that mortality among animals in the experimental group is not related to Y. pestis infection.

In the control group 2 all 10 animals died following the exposure.

Discussion

Antigen-specific response develops gradually. As shown in earlier studies lymphocytes with antigen-specific receptors appear first following the exposure to vaccination or infections, while the development of antibodies is usually delayed and happens during the effector phase of the antigen-specific immune response (Deryabin et al., 1993; Karalnik et al., 2001). Our experiment involving rabbits vaccinated with EV vaccine showed earlier appearance of antigen-specific lymphocytes compared to the appearance of antibodies.

Comparative analysis of the influence of betaleukin on the early antigen-specific response efficacy (appearance of LR-F1) and the antibody response on immunization of rabbits with live plague vaccine showed the correlation between these two stages. Faster appearance and disappearance of F1-specific lymphocytes lead to a faster and a more intensive antibody response. These results correlate with the results of the earlier studies on the influence of immunomodulators on the efficacy of herpetic inactivated vaccine showed (Omarova et al., 2011; Karalnik and Denisova, 2011). It is possible that LR-F1 play a regulatory role during the effector stage of antigen-specific response. Results of our study confirm that immunomodulation can be successfully used to assess the relations between different stages of antigen-specific response, and the regulatory role of LR-F1.

Antibody response observed in both experiments demonstrates high potential of betaleukin to increase immunogenic and protective efficacy of the live plague vaccine. The use of betaleukin alone does not have any protective effect from Y. pestis infection.

The use of a recombinant interleukin-1beta as an immunoadjuvant increases vaccination efficacy. In this experiment we were able to demonstrate that this product strengthens the effector phase of the immune response and also promotes early stage of antigen-specific immune response to EV vaccine.

Conclusion

Recombinant interleukin-1beta used during vaccination of rabbits with EV vaccine is able to stimulate both the early antigen—specific immune response by promoting appearance and disappearance of lymphocytes bearing receptors specific to F1 Y. pestis, and the antibody response to F1 Y. pestis.

The experiment in rabbits showed that the overall intensity of the effector stage of the immune response to EV vaccine is increasing with acceleration of the initial phase of antigen-specific response.

Combination of the live plague vaccine and the recombinant interleukin-1beta (betaleukin) substantially increased the immunogenic and protective efficacy of immunization in animal models using guinea pigs.

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