INDICES OF NON-SPECIFIC CELLULAR IMMUNE RESPONSE IN PIGS AFTER INTRADERMAL VACCINATION WITH DELETED AUJESZKY’S DISEASE VACCINE AND AFTER EXPERIMENTAL INFECTION

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Abstract

The kinetics of non-specific cellular immune response indices in pigs vaccinated intradermally (i.d.) versus intramuscularly (i.m.) with deleted Aujeszky’s disease (AD) vaccine and challenged with virulent strain of Herpesvirus suis type 1 (SHV-1) was evaluated. Three equal groups of 8-week-old piglets were used. The first two groups were vaccinated twice, 4 weeks apart, with attenuated vaccine at a dose of 2.0 ml (10^6.0 TCID50) I.M. (group I) or 0.2 ml (10^5.0 TCID50) I.D (group II) using the needleless apparatus. The control pigs were given 2.0 ml of PBS I.M. (group K). Seventy days after the first vaccination, a challenge test was done by instilling 0.5 ml of virulent Northern Ireland Aujeszky-3 (NIA-3) strain of SHV-1 (10^5.5 TCID50) into each nostril of all pigs. The following indices of non-specific cellular immune response after vaccination and experimental infection were evaluated: proliferation of lymphocytes stimulated by mitogens ConA and LPS (MTT test), metabolic activity of MN and PMN cells after stimulation by PMA (RBA test), and potential killing activity of MN and PMN cells (PKA test). The obtained results indicate that in the range of evaluated immunological indices the i.d. route of administration of deleted AD vaccine can be an alternative and can be utilized in the implementation of “vaccination – eradication programme” in the place of or parallelly to traditional i.m. route.

Key words: pigs, Aujeszky’s disease, intradermal vaccination, non-specific cellular immunity

Intensive implementation of Aujeszky’s disease (AD) eradication programme, based on deleted vaccines (“vaccination-eradication programme”) (24, 35, 36), has started at the turn of 80s-90s of the last century in many EU countries and in the United States (22, 33). It consists in mass, programmed for many years vaccinations of reproductive and fattening pigs. However, in fatteners the traditionally applied intramuscular (i.m.) vaccinations take a lot of time and work and are often connected with unfavourable local reactions, which are caused by bacterial infections as well as adjuvants contributing also to the spread of various infections. A considerable improvement of vaccination procedures and elimination of i.m. shortcomings can be obtained by intradermal (i.d.) vaccine application using a special needleless apparatus (2, 3, 37, 39).

The trials of i.d. immunizations against various infectious diseases were undertaken in many animal species - cattle, sheep, pigs, foxes and laboratory animals (12, 23). This vaccination route against AD was described for the first time by Vannier and Cariolet (38).

In case of i.d. vaccinations in pigs it is very important to acquire an adequate level of postvaccinal immune response. The skin, apart from protective functions, constitutes a very important part of immunological system (9, 18). The composition of skin immune system (SIS) consists of various epidermal and skin cells, qualified together as a skin associated lymphoid tissue (SALT), the most important of which are keratinocytes, skin dendritic cells, Langerhans cells, endothelial cells, T lymphocytes, as well as macrophages, neutrophils, mastocytes and melanocytes (10, 12, 15, 16, 18, 40).

The coordination of the function of these cells allowing SIS for quick and effective response to the delivered antigens causes that the i.d. route of vaccination seems to be a good alternative for the majority of traditional parenteral and intranasal routes (7, 12, 17, 21, 34, 37).

The aim of the study was to evaluate the kinetics of indices of non-specific cellular immune response in pigs vaccinated with deleted AD vaccine administered i.d. or i.m. and afterwards experimentally infected with virulent strain of Herpesvirus suis type 1 (SHV-1).
Material and Methods

Animals and vaccination. The study was performed on 21 piglets, 8-week-old, free from anti-SHV-1 antibodies, divided into three equal groups. The first two groups were vaccinated twice, 4 weeks apart, with deleted vaccine Porcilis Begonia (Intervet Int., the Netherlands) at a dose of 2.0 ml/pig (10^6.0 TCID50) i.m. (group I) or 0.2 ml/pig (10^5.0 TCID50) i.d. (group II). The control pigs were given 2.0 ml/pig of PBS i.m. (group K). Vaccinations by i.d. route were performed using a needleless apparatus SERENA, model SD 1-2 (Emaplast, Italy). Porcilis Begonia is a deleted, live attenuated vaccine based on gE- and TK-negative deletion mutant Begonia collected from the wild-type Northern Ireland Aujeszky-3 (NIA-3) strain of SHV-1. One vaccine dose contains at least 10^6.0 TCID50 of the virus. The vaccine was diluted ex tempore in adjuvantive diluent Dilucav Forte, based on α-tocopherol.

Challenge test. Seventy days (d) after the first vaccination all the pigs were intranasally infected with a dose of 10^3.0 TCID50 of virulent NIA-3 strain of SHV-1, kindly provided by the National Veterinary Research Institute in Pulawy, by instilling 0.5 ml of virus suspension into each nostril.

Indices of non-specific cellular immune response. Two blood samples from cranial caval vein were collected from each pig by a vacuum system (Sarstedt). One sample of about 6 ml was taken to the test-tube without anticoagulant to obtain sera. After blood coagulation the sample was centrifuged for 10 min at 3000 x g. The second sample of about 4 ml was taken to the heparinized test-tube.

The lymphocyte proliferative response (MTT test) as well as metabolic activity and potential killing activity of mononuclear (MN) and polymorphonuclear (PMN) cells (RBA and PKA tests) were evaluated. Samples were taken immediately before the 1st vaccination and after 2 weeks, immediately before the 2nd vaccination and after 2 and 4 weeks, on the day of experimental infection and then 3, 5, 7, 10 and 14 d post infection (d.p.i.).

MTT test. The proliferative response of blood lymphocytes stimulated by mitogens was performed by colorimetric method based on salt of tetrazolium MTT according to Mosmann (20) in modification of Siwicki et al. (29). Samples of peripheral blood were diluted in a ratio 1:1 with RPMI 1640 medium (Sigma, Germany). Lymphocytes were isolated at gradient of Histopaque 1077 (Sigma). Isolated lymphocytes (1-5 x 10^6) were suspended in RPMI 1640 with 10% foetal calf serum (FCS, Sigma) and poured into 96-well plates (NUNC) at amount of 100 µl/well. Each well was filled with 100 µl of mitogen: concanavalin A (ConA, Sigma) at concentration of 5 mg/ml PBS was added to each well and once more incubated for 4 h at 37°C (5% CO2). Then the plates were centrifuged for 5 min at 800 x g, the supernatant was removed, and 100 µg of dimethylsulfoxide (DMSO, Polish Chemical Reagents S.A.) were added to the wells. After 10 min, reading of absorbance was performed in microreader MRX 1.1 (Dynex, Great Britain) at wavelength 620 nm.

RBA test. Metabolic activity of blood MN and PMN cells was evaluated using RBA (Respiratory Burst Activity) test after cell stimulation by PMA (Phorbol Myristate Acetate, Sigma) described by Secombes (27) in modification by Siwicki et al. (30). Whole blood at amount of 100 µl was poured into 96-well plates (NUNC), next RPMI 1640 with 0.1% FCS (Sigma) at amount of 100 µl/well was added to each well and incubated for 24 h at 4°C. After incubation, the non-adhered cells were removed by drawing off a fluid and replaced by addition of 100 µl/well 0.1% dilution of NBT in RPMI 1640 or 0.1% dilution of NBT in RPMI 1640 with PMA – at amount of 1 µl/ml 0.1% dilution of NBT. The plates were incubated for 30 min at 37°C. After incubation and medium removing the cells were washed three times with 70% ethyl alcohol. Production of H2O2 by the cells was measured in microreader MRX 1.1 (Dynex) at wavelength 620 nm.

PKA test. Potential killing activity of blood MN and PMN cells was measured by spectrophotometric method with application of PKA (Potential Killing Activity) test according to Rook et al. (25) in modification by Siwicki and Anderson (28). Whole blood at amount of 100 µl was poured into 96-cell plates (NUNC), next RPMI 1640 with 0.1% FCS (Sigma) at amount of 100 µl/well was added to each well and incubated for 24 h at 4°C. After incubation the non-adhered cells were removed by drawing off a fluid and replaced by 0.1% dilution of NBT in PBS containing the 18 h culture of Staphylococcus aureus and afterwards incubated for 30 min at 37°C. After incubation and medium removing the cells were washed three times with 70% ethyl alcohol. The plates were dried for 30 min and next 120 µl of 2M KOH and 140 µl of DMSO were added to dissolve formazon. Reading was done in microreader MRX 1.1 (Dynex) at wavelength 620 nm.

Statistical evaluation. The obtained results were statistically elaborated by analysis of variance for comparison of several means (NIR test) at P < 0.05 and P < 0.01 with determination of standard deviations.

Results

The kinetics of variations in the proliferative response of T and B lymphocytes stimulated by mitogens ConA and LPS after vaccinations and after experimental infection is presented in Tables 1 and 2. As for proliferative response of T lymphocytes, a higher response was found in both vaccinated groups compared to group K 2 weeks after the 1st vaccination and in group i.d. compared to groups i.m. and K 4 weeks after the 1st vaccination and the differences were statistically significant.
Table 1
Influence of the route of vaccination with attenuated vaccine and NIA-3 SHV-1 experimental infection on the proliferative response of T lymphocytes stimulated by ConA

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; vacc.</th>
<th>2 weeks after 1&lt;sup&gt;st&lt;/sup&gt; vacc.</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; vacc.</th>
<th>2 weeks after 2&lt;sup&gt;nd&lt;/sup&gt; vacc.</th>
<th>4 weeks after 2&lt;sup&gt;nd&lt;/sup&gt; vacc.</th>
<th>challenge</th>
<th>3 d.p.i.</th>
<th>5 d.p.i.</th>
<th>7 d.p.i.</th>
<th>10 d.p.i.</th>
<th>14 d.p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (i.m.)</td>
<td>X</td>
<td>0.33</td>
<td>0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.35</td>
<td>0.19</td>
<td>0.22</td>
<td>0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.06</td>
<td>0.15</td>
<td>0.07</td>
<td>0.13</td>
<td>0.07</td>
<td>0.04</td>
<td>0.07</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>II (i.d.)</td>
<td>X</td>
<td>0.30</td>
<td>0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32</td>
<td>0.19</td>
<td>0.25</td>
<td>0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.08</td>
<td>0.09</td>
<td>0.15</td>
<td>0.07</td>
<td>0.08</td>
<td>0.09</td>
<td>0.08</td>
<td>0.03</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>K</td>
<td>X</td>
<td>0.27</td>
<td>0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25</td>
<td>0.18</td>
<td>0.20</td>
<td>0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.06</td>
<td>0.08</td>
<td>0.08</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.11</td>
<td>0.07</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Explanations: differences between groups A, B at P < 0.01; a, b at P < 0.05; d.p.i. – days post infection

Table 2
Influence of the route of vaccination with attenuated vaccine and NIA-3 SHV-1 experimental infection on the proliferative response of B lymphocytes stimulated by LPS

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; vacc.</th>
<th>2 weeks after 1&lt;sup&gt;st&lt;/sup&gt; vacc.</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; vacc.</th>
<th>2 weeks after 2&lt;sup&gt;nd&lt;/sup&gt; vacc.</th>
<th>4 weeks after 2&lt;sup&gt;nd&lt;/sup&gt; vacc.</th>
<th>challenge</th>
<th>3 d.p.i.</th>
<th>5 d.p.i.</th>
<th>7 d.p.i.</th>
<th>10 d.p.i.</th>
<th>14 d.p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.m.</td>
<td>X</td>
<td>0.21</td>
<td>0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26</td>
<td>0.21</td>
<td>0.27</td>
<td>0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52</td>
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<tr>
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<td>SD</td>
<td>0.10</td>
<td>0.11</td>
<td>0.16</td>
<td>0.06</td>
<td>0.08</td>
<td>0.10</td>
<td>0.06</td>
<td>0.15</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>i.d.</td>
<td>X</td>
<td>0.28</td>
<td>0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24</td>
<td>0.19</td>
<td>0.25</td>
<td>0.30</td>
<td>0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.04</td>
<td>0.22</td>
<td>0.12</td>
<td>0.05</td>
<td>0.05</td>
<td>0.07</td>
<td>0.06</td>
<td>0.26</td>
<td>0.12</td>
<td>0.07</td>
</tr>
<tr>
<td>K</td>
<td>X</td>
<td>0.23</td>
<td>0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22</td>
<td>0.15</td>
<td>0.22</td>
<td>0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.12</td>
<td>0.16</td>
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<td>0.02</td>
<td>0.04</td>
<td>0.16</td>
<td>0.08</td>
<td>0.11</td>
<td>0.11</td>
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</table>

Explanations: see Table 1
Table 3
Influence of the route of vaccination with attenuated vaccine and NIA-3 SHV-1 experimental infection on the metabolic activity of MN and PMN cells qualified by respiratory burst activity stimulated by PMA

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>1st vacc.</th>
<th>2 weeks after 1st vacc.</th>
<th>2nd vacc.</th>
<th>2 weeks after 2nd vacc.</th>
<th>4 weeks after 2nd vacc.</th>
<th>challenge</th>
<th>3 d.p.i.</th>
<th>5 d.p.i.</th>
<th>7 d.p.i.</th>
<th>10 d.p.i.</th>
<th>14 d.p.i.</th>
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<tbody>
<tr>
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<td>0.93</td>
<td>0.99</td>
<td>0.75</td>
<td>0.65</td>
<td>0.57 &lt;sup&gt;A&lt;/sup&gt;</td>
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<td>0.95</td>
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<tr>
<td></td>
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<td>0.33</td>
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<td>0.16</td>
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<td>0.19</td>
<td>0.28</td>
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</tr>
<tr>
<td>II (i.d.)</td>
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<td>2.04 &lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.84</td>
<td>0.76</td>
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<td>0.62 &lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.22</td>
<td>1.14</td>
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</tr>
<tr>
<td></td>
<td>SD</td>
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<td>0.22</td>
<td>0.41</td>
<td>0.29</td>
<td>0.34</td>
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<td>0.17</td>
<td>0.41</td>
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<td>0.38</td>
</tr>
<tr>
<td>K</td>
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<td>1.08 &lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.07</td>
<td>0.95</td>
<td>0.54</td>
<td>0.63</td>
<td>0.99 &lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.37</td>
<td>0.83</td>
<td>0.72 &lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>SD</td>
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<td>0.09</td>
<td>0.14</td>
<td>0.19</td>
<td>0.21</td>
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<td>0.13</td>
<td>0.24</td>
<td>0.16</td>
<td>0.39</td>
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</table>

Explanations: see Table 1

Table 4
Influence of the route of vaccination with attenuated vaccine and NIA-3 SHV-1 experimental infection on the potential killing activity of MN and PMN cells

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>1st vacc.</th>
<th>2 weeks after 1st vacc.</th>
<th>2nd vacc.</th>
<th>2 weeks after 2nd vacc.</th>
<th>4 weeks after 2nd vacc.</th>
<th>challenge</th>
<th>3 d.p.i.</th>
<th>5 d.p.i.</th>
<th>7 d.p.i.</th>
<th>10 d.p.i.</th>
<th>14 d.p.i.</th>
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<td>0.37</td>
<td>0.53 &lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.80</td>
<td>0.73</td>
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<tr>
<td></td>
<td>SD</td>
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<td>0.22</td>
<td>0.12</td>
<td>0.11</td>
<td>0.18</td>
<td>0.13</td>
<td>0.14</td>
<td>0.21</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>II (i.d.)</td>
<td>X</td>
<td>1.10</td>
<td>1.70</td>
<td>0.72</td>
<td>0.70</td>
<td>0.50</td>
<td>0.41</td>
<td>0.38 &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.93</td>
<td>0.88</td>
<td>1.06</td>
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<tr>
<td></td>
<td>SD</td>
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<td>0.43</td>
<td>0.25</td>
<td>0.25</td>
<td>0.19</td>
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<tr>
<td>K</td>
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<td>1.38</td>
<td>0.67</td>
<td>0.53</td>
<td>0.32</td>
<td>0.37</td>
<td>0.48</td>
<td>0.83</td>
<td>0.66</td>
<td>0.81 &lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>SD</td>
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<td>0.08</td>
<td>0.24</td>
<td>0.16</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Explanations: see Table 1
In the period after challenge on the 3rd d.p.i. we found statistically significant increase (P < 0.05) in proliferative activity of T lymphocytes in group II compared to groups I and K. On the 5th and 7th d.p.i. a statistically significant increase in proliferative activity of T lymphocytes in both vaccinated groups compared to group K was noticed, whereas in group I the activity increased on the 10th d.p.i. compared to controls.

As for proliferative response of B lymphocytes 2 weeks after the 1st vaccination higher activity was found in group I compared to groups II and K and 4 weeks after the 1st vaccination lower response in group K than in vaccinated groups and the differences were statistically significant. In the period after experimental infection we found already on the 3rd d.p.i. a higher (P < 0.05) proliferative activity of B lymphocytes in group I compared to group K and in both vaccinated groups compared to group K on the 5th d.p.i. and the differences were statistically significant. However, on the 7th d.p.i. the increase in proliferative activity of B lymphocytes in vaccinated groups compared to group K was in group I significantly higher at P < 0.05 and in group II at P < 0.01. On the 10th and 14th d.p.i. in spite of higher proliferative response in immunized groups, the statistically significant differences compared to group K were not found.

The formation of metabolic activity of MN and PMN cells evaluated by the level of respiratory burst activity (RBA) stimulated by PMA after vaccinations and after experimental infection is presented in Table 3.

In two weeks after the 1st immunization statistically significant increase in metabolic activity was found in group I compared to group II at P < 0.05 and to group K at P < 0.01. In other periods of postavaccinal response, statistically significant differences in metabolic activity between vaccinated groups and control group were not observed. However, in the period after experimental infection on d.p.i. 3, 10 and 14 the statistically significant increase of RBA levels was noticed in both vaccinated groups compared to group K.

The kinetics of variations in potential killing activity of MN and PMN cells evaluated by ability to intracellular killing after vaccinations and after experimental infection is presented in Table 4.

After vaccinations, no statistically significant differences between vaccinated and control groups were noticed in spite of an increase in potential killing activity in both vaccinated groups observed 2 weeks after the 1st vaccination. However, after experimental infection on the 3rd d.p.i. a statistically significant increase (P < 0.05) in PKA levels in group I compared to group II was observed. On the 10th d.p.i. a higher (P < 0.01) PKA level in group I compared to group K was noticed and the difference was statistically significant, too.

Discussion

In the early period of viral infection the body protection against spreading of the virus first of all relies on initiation of mechanisms of non-specific immunity. In cellular non-specific immune response, the phagocytic cells comprising monocytes/macrophages and neutrophils are of great importance (14). In the first stage of infection SHV-1 penetrates epithelial cells of nasal mucosa and afterwards reaches the deeper tissue layers confronting with macrophages and T lymphocytes (1). Macrophages take part in the induction of T lymphocytes response through presentation of antigen and secretion of many various cytokines. They also demonstrate direct cytotoxicity towards cells infected with the virus (4). Clinical picture of SHV-1 infection, accompanied by high fever and losses of body weight, suggests that similarly to other herpesvirus infections the fundamental role in the pathogenesis of disease is played by proinflammatory cytokines, mainly IL-1 and TNF-α, produced by activated macrophages and neutrophils (4, 31). In our study, the increase in metabolic and killing activities of MN and PMN cells after challenge with virulent SHV-1 strain corresponded to appearance of clinical signs in infected animals. On the 3rd and 5th d.p.i. the higher activity was found in control group, where intense clinical signs appeared at this time. However, in groups of vaccinated pigs, where clinical signs were decidedly weaker, the increase in neutrophils and monocytes activities occurred much later i. e. after the 7th d.p.i.

In the body response to vaccination against AD and experimental infection with virulent SHV-1 the mechanisms of cellular immunity fulfill extremely significant function (4, 19). Estimation of significance of cellular immunity in the course of AD was influenced by demonstration of its importance in the prophylaxis of other herpesvirus infections, e. g. Herpes simplex in people (13, 42) and BHV-1 in cattle (5, 26). In our study, the proliferative activity of lymphocytes stimulated by mitogens was evaluated after vaccination and challenge test. In both vaccinated groups the increase in proliferative activity of T lymphocytes as a response to mitogen ConA was observed from the 2nd week after the 1st vaccination followed by gradual decrease to the moment of experimental infection, what clearly confirms the stimulation of mechanisms of cellular immunity due to vaccination. The proliferative activity of B lymphocytes stimulated by LPS was formed in a similar manner. The most intensive response was observed in vaccinated groups 2 and 4 weeks after the 1st vaccination, but in group I the increase in proliferative activity in the 2nd week after experimental infection was significantly higher than in group II, which correlates with higher level of SN antibodies found at that time in group I. De Bruin et al. (6) observed the increase in proliferative activity of T and B lymphocytes stimulated in vitro by SHV-1 after vaccination of pigs with avirulent M141 strain. However, after experimental infection with virulent SHV-1 strain these authors found the increase in proliferative activity only of T lymphocytes, but did not observe changes in proliferation of B lymphocytes. Vanderpotten et al. (37), who compared the proliferative response of lymphocytes stimulated by SHV-1 after vaccination by i.m. and i.d. routes, obtained higher
proliferation in groups of pigs vaccinated i.d., although these results were not correlated with better protection of these groups after the challenge test.

In our study, 2 weeks after the 1st vaccination the statistically significant increase \((P < 0.05 \text{ and } P < 0.01)\) in metabolic activity of MN and PMN cells isolated from peripheral blood was also found, as well as their slightly higher killing activity in both vaccinated groups compared to the control group. This clearly confirms the intensity of immunological reactions taking place at this time. MN and PMN cells play an extremely important role in specific and non-specific body immune response for antigen. Besides the ability to synthesis and secretion of various substances participating, among other things, in stimulating lymphocyte and other cell proliferation, as well as antigen presentation, they also show the ability to phagocytosis, cytotoxicity and cytolysis (8). It is supposed that higher activity of monocytes and neutrophils in group vaccinated i.m. compared to group vaccinated i.d. observed 2 weeks after the 1st vaccination was caused by interception of Langerhans cells present in the skin, extremely effective in catching and presenting of antigen, the part of function of monocytes connected for example with antigen presentation. As a result of contact with antigen they undergo an activation and maturation, migrate from antigen presentation. As a result of contact with antigen they undergo an activation and maturation, migrate from antigen presentation. The blood vessel endothelial cells to participate in the cell communication and enable skin fibroblasts and growth Factor) \((15, 18, 32)\). Cytokines mediate in the place of or parallelly to traditional i.m. route of administration of deleted vaccine against AD can be an alternative and can be utilized in the implementation of “vaccination-eradication programme” in the place of or parallelly to traditional i.m. route.

Keratinocytes are also an extremely important element of SIS constituting the first line of defense. Their function first of all results from an ability to produce many cytokines and growth factors, playing the central role in mobilizing blood leukocytes and activating other skin cells e. g. they participate in differentiation and migration of Langerhans cells (32). They produce and release among others, IL-1α, IL-3, IL-6, IL-8, IL-10, IL-12, TNFa, TNFβ, and FGF (Fibroblast Growth Factor) \((15, 18, 32, 41)\). Cytokines mediate in cell communication and enable skin fibroblasts and blood vessel endothelial cells to participate in the immune response \((41)\).

In skin immune response many other types of cells take also part. Dendritic cells in the skin play similar function to Langerhans cells in epidermis. They catch an antigen, migrate to lymph nodes and present processed antigen to T lymphocytes. The blood vessel endothelial cells play, however, control functions towards leukocytes migrating outside blood vessels and also function of antigen presenting cells \((12, 41)\). Dendritic epidermal T cells (DETC) occurring on the skin take part in recognizing egzogenic and own antigens without participation of MHC particles and function as the first body defense line directed against pathogens \((41)\).

An infection with NIA-3 strain on the 3rd d.p.i. caused an increase of proliferative response of T lymphocytes for mitogen ConA in both vaccinated groups compared to the control. However, only in group vaccinated i.d. this increase was statistically significant, both compared to control group and to group vaccinated i.m. On other days until the end of the experiment, comparable high proliferative activity of T lymphocytes was observed in both immunized groups. The response of B lymphocytes to mitogen LPS was formed a little differently. From the moment of experimental infection a gradual increase in the activity of B lymphocytes was observed in all groups subjected to challenge, but this process was slightly more effective in group I than in group II. On the 3rd d.p.i. the values achieved in group vaccinated i.m. were significantly higher than in group vaccinated i.d., but already on the 7th d.p.i. no differences between examined vaccinated groups were found. The obtained results in relation to proliferative activity of T and B lymphocytes suggest that in pigs vaccinated by i.d. route, after experimental infection, the stimulation of cellular immune mechanisms connected with activation of T lymphocytes was induced better and quicker, compared to pigs vaccinated by i.m. route, where higher activity of B lymphocytes was observed in the first days after experimental infection. The above results can be explained by the fact that vaccine antigen administered i.d. in connection with a special efficiency of dendritic cells occurring on the skin to present antigens, make a strong impulse stimulating T lymphocytes to proliferation and differentiation into effector cells, and also into memory cells responsible for secondary immune reaction after repeated contact with the same antigen. Cited formerly De Bruin et al. \((6)\) noticed that infection with NIA-3 strain of pigs immunized earlier induced the increase in proliferative activity of T lymphocytes stimulated \(in vitro\) by SHV-1 with simultaneous lack of B lymphocytes stimulation. It made them conclude that secondary response of T lymphocytes had a decisive significance in the immune response in pigs experimentally infected with SHV-1.

The obtained results of our study in the range of evaluated immunological indices indicate that the i.d. route of administration of deleted vaccine against AD can be an alternative and can be utilized in the implementation of “vaccination-eradication programme” in the place of or parallelly to traditional i.m. route.

References

