Physiological and Immunological Profiles after Intrauterine Immunization

F. KOVÁŘŮ1, H. KOVÁŘŮ2, A. FIŠEROVÁ3, E. MATAŁÓVÁ4, P. ZELNÍČKOVÁ1, L. LANDA5, M. PALÍKOVÁ1

1University of Veterinary and Pharmaceutical Sciences, Brno, 2Charles University, 1st Faculty of Medicine, Prague, 3Institute of Microbiology, Academy of Sciences CR, Prague, 4Institute of Animal Physiology and Genetics, Academy of Sciences CR, Brno, 5Masaryk University, Faculty of Medicine, Department of Pharmacology, Brno, Czech Republic

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Abstract


Onset of physiological immunocompetence, i.e. ability to respond to antigen by production of specific antibodies without disturbance of the physiological integrity of the organism was studied in pig fetuses. Closed surgery technique was used to expose the fetuses. Flagellin in non-complete adjuvant was used as antigen. Fetuses were immunized on day 54 of gestation, i.e. in the period when T-B dichotomy in secondary lymphatic organs as principal sign of differentiation is already present. Besides the specific immunological response the intrauterine immunization resulted in increased numbers of agranulocytes within secondary lymphatic organs and in circulating blood. No maturation to their plasma cells but increased activity of gamma-glutamyl transpeptidase (GGT) was observed. Increased concentration of total proteins with predominant increase of albumins and gamma globulins along with a decrease of actual amount of alpha-1-fetoprotein was found in blood serum of immunized fetuses. Shift of maturation curves of erythroid and myeloid line to younger developmental forms appeared in haemopoietic organs of these fetuses. In older fetuses immunized on day 74 of gestation, i.e. when a basis of follicular organisation of lymphatic tissue is present, a typical cytological immune reaction “of adult type” was observed. It was characterized by multiplication of lymphocytes of all size categories and their differentiation to plasma cells.

Developmental immunology has an ideal experimental model in porcine fetuses because of the character of placentation in this species (placenta epitheliochorialis, diffuse type). This type of placenta not only prevents transfer of immunoglobulins between mother and fetus but it also markedly constrains the non-controllable antigenic stimulation during intrauterine development (Sterzl and Silverstein 1967; Kovářů et al. 1969). However, developmental immunology is focused not only on the study of spontaneous development of effector systems of immunity but it also takes advantage of possible induction of immune response at particular developmental stages under precisely defined conditions. For this purpose a unique ontogenetic model of development of physiological immunocompetence has been elaborated. It is based on the ability of developing fetuses to respond to specific antigenic stimulus after intrauterine immunization without affecting their basic physiological indices and integrity (Kovářů et al. 1971; Kovářů and Stožický 1986; Reháková et al. 1996).

The onset of physiological immunocompetence against sheep erythrocytes (corpuscular antigen) and φX phage 174 (model of viral antigen) on day 70 of gestation has been demonstrated (Sterzl and Kovářů 1877). The aim of this experiment was to study the antigenic stimulation during an earlier prenatal period (from day 54 of gestation), i.e. during...
the key developmental stages of spontaneous maturation of lymphatic and haemopoietic systems as a morphological substrate of immune responses. Changes in lymphatic and haemopoietic organs are reported as well as further basic physiological indices of fetuses after intrauterine immunization.

Materials and Methods

We used 8 pregnant minipig (Minnesota) sows in the second half of gestation (a total of 52 fetuses). Surgical intervention was carried out on days 54 and 74 of gestation. Immunization was carried out using a closed intrauterine immunization technique (Kováfû et al. 1971; Kováfû and Stožický 1986; Rehäuserová et al. 1996) in halothan-oxygen anesthesia with 50 percent N2O admixture (spontaneous breathing). Basic physiological indices (mean arterial pressure, respiratory rate, pulmonary ventilation, rectal temperature and values of pH, pO2, pCO2, and SO2) in maternal and fetal blood were continuously monitored. The above mentioned technique enabled the surgical intervention on pregnant uterus without disturbing the basic physiological integrity of mother or fetuses. Flagellin was used as a T-independent antigen (100 µg i.m. per fetus in non-complete adjuvant AL-SPAN-OIL adjuvans – Sevac, Praha). After intrauterine immunization half of the number of fetuses was kept in uterus without immunization as controls in each sow (26 fetuses immunized and 26 controls). Besides the classical haematological and quantitative cytological technique (Kováfû et al. 1995), the following methods were used: Immunochemical technique for demonstration of immunoglobulins using monospecific anti-µ, anti-γ or anti-α sera (Prokešová et al. 1979). Activities of Na+K+-ATPase, Ca2+-ATPase, Mg2+-ATPase and gamma-glutamyltranspeptidase (GGT) were determined in primary and secondary lymphatic organs as a biochemical criterion of cell membrane maturation (Kováfû et al. 1982; Kováfû et al. 1992). The techniques of electrophoresis, immunoelectrophoresis and rocket immunoelectrophoresis were used for analyses of proteins in serum and other body fluids.

Results and Discussion

As shown earlier, pig fetuses are able to respond to immunization with flagellin by production of specific antibodies as soon as from day 54 of gestation, and they are also able to recognize certain hapten groups (e.g. dinitrophenyl – DNP and fluorescein). This fact was detected at both serological and cellular levels (Tlaskalová-Hogenová et al. 1994). In this prenatal period T-B dichotomy in primary and secondary lymphatic organs is already well apparent. However, the numbers of immunocompetent B-cells with IgM receptor are rather low and they range about 1 × 10^6 which is 300 times less than in a newborn piglet (3 × 10^8) (Kováfû et al. 1995). Neither follicular and periarteriolar organization of secondary lymphatic organs is yet present in this period, and lymphocytes are loosely diffused in their histioreticular structure (Kruml et al. 1970). Despite this qualitative and quantitative handicap porcine fetuses on day 54 of gestation are already able to respond in a goal-directed way to antigen. Besides the production of specific antibodies (Tlaskalová-Hogenová et al. 1994) in this study polyclonal activation of synthesis of all

![Fig. 1. Effect of intrauterine immunization in FD54 on immunoglobulin levels in fetal blood serum seven days later (FD61)](image1)

![Fig. 2. Effect of intrauterine immunization in FD74 on immunoglobulin levels in fetal blood serum 12 days later (FD86)](image2)
immunoglobulin groups (IgM, IgG, IgA) in younger (Fig. 1) and in older fetuses (Fig. 2) was detected. It is accompanied by expression of IgG receptors on the surface of lymphocytes, that are not present in non-stimulated fetuses, and it is also accompanied by distinct expression of MHC antigen class II (Trebichavský 1985).

Fig. 3. Effect of intrauterine immunization in FD54 on maturation curve of erythroid cells in fetal liver 7 days later (FD61)

Fig. 4. Effect of intrauterine immunization in FD54 on maturation curve of myeloid cells in fetal liver 7 days later (FD61)

Fig. 5. Effect of intrauterine immunization in FD54 on mononuclear cell proportion in fetal liver 7 days later (FD61)

Fig. 6. Effect of intrauterine immunization in FD54 on mononuclear cell proportion in fetal spleen 7 days later (FD61)

Fig. 7. Effect of intrauterine immunization in FD54 on mononuclear cell proportion in blood 7 days later (FD61) - per 1mm³ of blood

Fig. 8. Effect of intrauterine immunization in FD54 on mononuclear cell proportion in blood 7 days later (FD61)
Intrauterine immunization had also a polyclonal effect on day 54 of gestation on the whole system of haemopoiesis and lymphopoiesis. This is observed especially in fetal liver that is the major haemopoietic organ in this period (Kováfiú et al. 1995). This was manifested by the shift in the peaks of maturation curves of erythroid (Fig. 3) and myeloid (Fig. 4) lines towards younger developmental forms. Concerning mononuclear cells, cytological characteristic of immune response in younger fetuses (54th day of gestation) is characterized by evident increase in actual and relative representation of lymphoblasts and medium lymphocytes as well as by various developing stages of monocytes. This characteristic was apparent in both haemopoietic (fetal liver) and secondary lymphatic (spleen) organs (Figs. 5 and 6). This characteristic was also apparent in circulating blood both in relative terms (Fig. 7) and expressed per 1 mm³ of blood (Fig. 8). In fetuses immunized on day 74 of gestation and studied 12 days later, i.e. in the period when follicular and periarteriotal organization of lymphatic tissue was already present, besides increased numbers of lymphocytes of particular size categories also maturation leading to plasma cells appeared. This was apparent in both lymph nodes and circulating blood (Fig. 9, 10).

Gamma-glutamyl transferase (GGT) activity was significantly higher (Tab. 1, 2), in immunized fetuses (54th day of gestation) in lymphocytes of secondary lymphatic organs.

<table>
<thead>
<tr>
<th>Lymphocytes from</th>
<th>n</th>
<th>GGT</th>
<th>Na⁺K⁺-ATPase</th>
<th>Mg²⁺-ATPase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>7</td>
<td>62 ± 8</td>
<td>48 ± 5</td>
<td>162 ± 14</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>7</td>
<td>45 ± 7</td>
<td>35 ± 4</td>
<td>145 ± 17</td>
</tr>
<tr>
<td>Thymus</td>
<td>7</td>
<td>41 ± 8</td>
<td>8 ± 2</td>
<td>38 ± 4</td>
</tr>
</tbody>
</table>

Table 1
Initial values of enzyme activities (control activity)

<table>
<thead>
<tr>
<th>Lymphocytes from</th>
<th>n</th>
<th>GGT</th>
<th>Na⁺K⁺-ATPase</th>
<th>Mg²⁺-ATPase</th>
<th>% control activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>10</td>
<td>165 ± 15</td>
<td>110 ± 12</td>
<td>92 ± 8</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>8</td>
<td>170 ± 18</td>
<td>101 ± 8</td>
<td>115 ± 13</td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>10</td>
<td>92 ± 12</td>
<td>104 ± 10</td>
<td>121 ± 17</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Effect of intrauterine immunization on enzyme activity of lymphocytes 7 days later (FD61)

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Fig. 9. Effect of intrauterine immunization in FD74 on lymphocyte proportion in lymph nodes 12 days later (FD86) - per 1 gram w. w.

Fig. 10. Effect of intrauterine immunization in FD74 on mononuclear cell proportion in blood 12 days later (FD86)
This corresponds with our previous conclusions, namely, a decreased degree of lipid fluidity (lipid microviscosity) in plasma membranes in lymphocytes of secondary lymphatic organs in immunized fetuses. This is in direct correlation with increased activity of GGT as markers of maturation of plasma membrane in these cells (Kováfiû et al. 1979). These findings are in agreement with our earlier results obtained in postnatal period in piglets kept under germ-free conditions or kept on a non-antigenic diet. Activity of GGT was significantly lower in lymphocytes of primary lymphatic organs in these piglets than in piglets kept under conventional conditions and exposed to antigenic pressure of microbial flora. On the other hand, GGT activity was highly increased in lymph node lymphocytes of immunized piglets.

Consequences of intrauterine immunizations are manifested in the protein composition of fetal serum. Apart from previously described increase in the individual immunoglobulin classes also changes in alpha-globulin fraction occur frequently. Increase in percentage of albumin in fetal serum occurred in immunized fetuses accompanied by increased total serum proteins. These findings for 54-day-old pig fetuses on days 7 and 14 after immunization are shown in Figs 11ab. From the point of view of immunoregulatory mechanisms a decrease in actual amount of alpha 1–fetoprotein in

Table 3
Effect of intrauterine immunization in FD54 on AFP levels in fetal body fluids 7 days later (FD61)

<table>
<thead>
<tr>
<th>sample</th>
<th>No exp</th>
<th>A</th>
<th>control</th>
<th>immunized</th>
<th>% of control</th>
<th>range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood serum</td>
<td>7</td>
<td>24</td>
<td>3.6</td>
<td>2.7</td>
<td>75</td>
<td>60-78</td>
</tr>
<tr>
<td>amniotic fluid</td>
<td>4</td>
<td>15</td>
<td>0.52</td>
<td>0.33</td>
<td>64</td>
<td>52-70</td>
</tr>
<tr>
<td>allantoic fluid</td>
<td>4</td>
<td>15</td>
<td>0.61</td>
<td>0.38</td>
<td>63</td>
<td>30-75</td>
</tr>
</tbody>
</table>

Fig. 11a. Effect of intrauterine immunization in FD54 on albumin concentration in fetal blood serum 7 and 14 days later

Fig. 11b. Effect of intrauterine immunization in FD54 on total protein concentration in fetal blood serum 7 and 14 days later

Fig. 12. Effect of intrauterine immunization in FD54 on cortisol concentration in fetal blood serum 7 and 14 days later
immunized fetuses is of particular interest. This is apparent not only in fetal serum but also in amniotic and allantoid fluid (Tab. 3). Since this oncofetal glycoprotein shows a significant immunosuppressive effect in vitro (Kovář, 1978; Kovář et al., 1976), its decrease in body fluids in vivo during immune response suggests its possible participation in the regulation of development of immunocompetence during ontogenesis.

Our results indicate that the consequences of intrauterine immunization are manifested not only by production of specific antibodies but they are also reflected in other organ systems due to the complicated compensatory mechanisms of neuroimmunoendocrine regulation (Kovář et al., 1997). In order to eliminate the stress effect of intrauterine immunization in the developing fetuses we determined their serum cortisol concentrations on days 7 and 14 after immunization. In stimulated fetuses its increase was not significant, and its actual values did not indicate the presence of the classical stress reaction in the cascade of immunomorphological and physiological changes following intrauterine immunization (Fig. 12). Study of possible late consequences of intrauterine immunization in the early postnatal period based on changes in physiological and immunological indices in stress-affected animals (as a model of intrauterine infection) will be the aim of our further research.

Acknowledgement
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References

Změny fyziologických a imunologických ukazatelů po intrauterinní imunizaci


U starších fetů imunizovaných 74. den gestace, kdy jsou již vytvořeny základy folikulárně organizované lymfatické tkání můžeme pozorovat typicky cytologickou imunitní reakci „dospělého typu“ charakteristickou zmnožením lymfocytů všech velikostních kategorií a jejich diferenciaci až do buněk plazmatických.
KOVAŘÍČ, H., KOVAŘÍČ, F., HASKOVÁ, V. 1997: Cell surface enzymes of brain cortex cell or lymphocytes during early allogeneic reaction. Physiol Res 46: 137-144
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Fig. 1. Immunohistochemical localization of IgG in maternal part of intact pig placenta (indirect immunofluorescence antiserum labeled with FITC). No transfer of immunoglobulin from mother to fetuses.
Fig. 2. Immunohistochemical localization of IgG in maternal part of pig placenta seven days after surgical intervention (indirect immunofluorescence antisera labeled with FITC). No transfer of immunoglobulin from mother to fetuses.