ACTIVITY OF T AND B LYMPHOCYTES AND BLOOD PHAGOCYTES IN PIGS IMMUNOMODULATED WITH BIOIMMUNO AND/OR IMMUNISED WITH RESPISURE ONE VACCINE AGAINST MYCOPLASMAL PNEUMONIA OF SWINE

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Abstract

The purpose of the studies was to determine the proliferation activity of T and B lymphocytes as well as the metabolic and potential killing activity of blood phagocytes in pigs after the immunomodulation with the Bioimmuno preparation and/or immunisation with the ‘Respisure One’ vaccine against mycoplasmal pneumonia of swine. The studies were performed on piglets at the age of 4 weeks, divided into four groups of seven animals each. The biopreparations were administered according to the following pattern: Bioimmuno (1 kg/50 kg of feedstuff) for 48 h before vaccination with Respisure One (2 ml/animal i.m.) on the 28th d of life (group I), Bioimmuno (1 kg/50 kg of feedstuff) on days 26 and 27 of life (48 h before vaccination with Respisure One of groups I and III) (group II), Respisure One on the 28th d of life (group III) and PBS (2 ml/animal i.m.) simultaneously with vaccination of groups I and III (group C – control). The proliferation activity of T and B lymphocytes (MTT test) and the metabolic and potential killing activity of the phagocytes (RBA and PKA tests) were determined in whole blood. Statistically significantly higher (P<0.05) proliferation activity of Con A-stimulated T lymphocytes as well as LPS-stimulated B lymphocytes were found in the stimulated and immunised group I and the immunised group III in comparison with the stimulated group II and group C. Higher metabolic and potential killing activities of the phagocytes were also detected in all experimental groups compared with the control one. The studies have demonstrated that the Bioimmuno immunomodulator and/or the Respisure One vaccine stimulate the proliferative response of T and B lymphocytes, as well as they increase the metabolic and potential killing activity of blood phagocytes, contributing to the improvement in the immune system functioning and to the protection of the swine organism against the infections of the respiratory system.

Key words: pig, mycoplasmal pneumonia of swine, Bioimmuno, Respisure One, non specific immunity.

Nowadays, the greatest problem for pig producers all over the world, among the disorders of the respiratory system, is not the occurrence of monoetiological infections, but more and more common poliaetiological infections. During their course, several other species of microorganisms, apart from Mycoplasma hyopneumoniae (Mhp) are simultaneously, very frequently isolated from lung lesions (27). To a considerable degree, it hinders the unanimous identification of the primary aetiological factor. The multifactorial pathogenic condition, in which difficulties to perceive and eliminate clinical syndromes of the respiratory system are indicated, is called porcine respiratory disease complex - PRDC (25, 27, 36). The prevention of the PRDC requires the provision of adequate environmental conditions for animals and possibly high efficiency of their immune system (35).

Due to the large diversity of aetiological factors, a suitable vaccination programme providing for the protection of the respiratory system against PRDC is difficult to select. Vaccinations are conducted within the whole technological groups of animals, without taking into consideration singular individuals, which are potentially not fit for the vaccinations because of their bad health condition (26). The influence of environmental and stressful factors has an adverse effect on non-specific defence mechanisms and specific immunity in animals. Therefore, some hopes are pinned on immunomodulators, which intensely activate both non-specific and specific cellular and humoral defence mechanisms. They are able to increase the metabolic and phagocytic activity of neutrophils and monocytes/macrophages as well as the activity of T and B lymphocytes, including enhancement of the production of specific antibodies by plasmatic cells. Simultaneously, they strengthen the synthesis and excretion of interferons, lysozyme and other substances conditioning humoral immunity. In such a manner, it is possible to obtain high level of anti-infectious immunity, which allows to maintain a good health status in swine herds and to reduce considerably the losses caused by...
infectious diseases of the respiratory system (3, 13, 19, 20).

A number of substances stimulating the immune system have been applied in veterinary medicine, but the leading role is played by non-specific immunomodulators with the multifactorial mechanism of functioning. An important group among natural immunomodulators is highly polymerised polysaccharides extracted from fungi – glucans and mannans (8, 20). For example, β-D-glucan, termed lentitin, isolated from Lentinus edodes fungus has an inducing influence on the activity of NK cells, macrophages, and their ability to produce cytokines. Glucans (1.3/1.6-β-glucan) isolated from Saccharomyces cerevisiae, yeast, given to animals, increase the immunity against the experimental fungal, viral, and bacterial infections, and stimulate antiviral immunity as well. It has also been indicated that the oral administration of baker’s yeast (Saccharomyces cerevisiae) reduces the incidence of the respiratory system diseases in mice caused by Klebsiella pneumoniae and Streptococcus pyogenes (2). In the experimental studies, a stimulating influence of the glucan on phagocytosis, activation of the complement, and induction of interferon production has been confirmed in pigs infected with SIV and its adjuvant effect on the generation of antibodies has been also demonstrated (1, 8, 10).

In the group of synthetic immunomodulators, isoprinosine (methisoprinol, inosine pranobex) presents a beneficial stimulating activity. In medicine, it is used as an immunostimulator and a drug in the elimination of some viral infections. In the in vitro studies, it has been demonstrated that the compound intensifies the influence of mitogens (PHA, Con A) on the T lymphocyte proliferation and stimulates humoral and cellular immunity, as well as non-specific defence mechanisms intracorporally. Isoprinosine has the ability to activate macrophages, to stimulate phagocytosis, to strengthen the maturation of T lymphocytes, and to stimulate the activity of CD4+ and CD8+ lymphocytes as well as NK towards infected cells (23, 28, 30). Owing to the regulative influence on the activity of helper and suppressor T lymphocytes, it has also an indirect effect on the organism humoral response. Moreover, it is capable of stimulating the maturation of B lymphocytes and synthesising specific anti-viral antibodies (23). The beneficial influence on the health status as well as haematological and immunological indices of the combined administration of isoprinosine and iron preparations in the prophylaxis of anaemia in piglets (15, 16, 18) has been demonstrated together with the immunostimulative effect on the immune system cells in piglets during the neonatal period (9), as well as the lack of toxic and immunosuppressive effect even after the administration of heavy doses (4, 11).

The purpose of the studies was to determine the proliferation activity of T and B lymphocytes as well as the metabolic and potential killing activity of blood phagocytes in pigs after the immunomodulation with the Bioimmuno preparation and/or immunisation with the Respisure One vaccine.

Material and Methods

Animals. The studies were performed at a pig producing farm, with the basic herd consisting of 16 sows, in which the production of their own fatteners, and the one based on piglets from the purchase, amounted to about 600 animals annually. The weaning of piglets took place between the 28th and 35th d of their life. No specific immunoprophylaxis against mycoplasmal pneumonia of swine (MPS) had been used on the farm, whereas only vaccinations against colibacteriosis were conducted. Lung lesions characteristic for MPS found in post-slaughter examinations were the reason to choose this farm to realise the experiment. During the experiment, the conditions of pig nutrition and maintenance were identical. The pigs were fed ad libitum full-portioned granulated feedstuff, with the possibility of the evaluation of its consumption per animal/daily. The rooms for pigs were equipped with the system of mechanical ventilation with manual control provided for good environmental conditions.

The studies were carried out on piglets of both genders, with the similar number of young boars and gilts and the initial body mass of about 7-8 kg (the 28th d of life), divided into four groups of seven animals.

Biopreparations. In the experiment we used: inactivated vaccine against MPS - Respisure One (Pfizer Inc. Animal Health Group) – each dose of the vaccine (2 ml) contained NL 1042 Mhp 4.5–5, 2 log10 RP strain and Amphigen oil adjuvant and the Bioimmuno preparation (Inland Fisheries Institute in Olsztyn, Poland) containing 40 g of methisoprinol + 960 g of Saccharomyces cerevisiae to be given with feedstuff.

The biopreparations were administered according to the following pattern: group I - Bioimmuno with feedstuff, at a dose of 1 kg/50 kg of feed for 48 h before pigs’ immunisation with Respisure One on the 28th d of life; group II – Bioimmuno only, administered with feedstuff at a dose of 1 kg/50 kg of feed for 48 h before immunisation with Respisure One of groups I and III; group III – immunisation with the Respisure One vaccine on the 28th d of life, at a dose of 2 ml/animal i.m., and group C (control) – the PBS administration, at a dose of 2 ml/animal i.m. simultaneously with immunisation of groups I and III.

Immunological assays. For the determination of the non-specific immunity indices, a 4-ml sample of blood from the anterior vena cava was collected from each pig into the heparinised tube. The samples were collected on the –2nd, 0, 3rd, 7th, 14th, 21st, and 42nd d after immunomodulation and/or immunisation. The proliferation activity of T and B lymphocytes (MTT test) and the metabolic and potential killing activity of blood phagocytes (RBA and PKA tests) were determined in whole blood.

The proliferation test of T and B lymphocytes stimulated by mitogens ConA and LPS, respectively, was performed by means of the colorimetric method
isolated lymphocytes (1–5×10^6) were suspended in 1640 medium (Sigma, Germany). Lymphocytes were
Blood samples were diluted in a ratio of 1:1 using RPMI
DMSO were added in order to dissolve formozan. The
30 min and then, 120 µl of 2M KOH and 140 µl of
tetrazolium bromide, Sigma) at concentration of 5
solution (3-[4,5-dimethylthiazol-2-yl]-5,2diphenyl
tetrazolium salt MTT according to
Based on the tetrazolium salt MTT according to
Mosmann (22) in modification by Siwicki et al. (33).
Blood samples were diluted in a ratio of 1:1 using RPMI
1640 medium (Sigma, Germany). Lymphocytes were
isolated in Histopaque 1077 gradient (Sigma). The
isolated lymphocytes (1–5×10^6) were suspended in
RPMI 1640 medium with the addition of 10% foetal calf
serum FCS (Sigma) and poured into the 96-well plates
(NUNC) at amount of 100 µl/well. Hundred microlitres
of mitogen: concanavalin A (Con A, Sigma) at
concentration of 64 µg/mL, or lipopolysaccharide (LPS, Sigma) extracted from Escherichia coli
serotype 0111:B4 at concentration of 160 µg/mL was added into
each well. RPMI 1640 medium at amount of 100 µl/well
was used as control. The plates were incubated for 72 h
at 37ºC (5% CO₂). After incubation, 10 µl of MTT
solution (3-[4,5-dimethylthiazol-2-yl]-5,2diphenyl
tetrazolium bromide, Sigma) at concentration of 5
mg/mL PBS was added into each well and incubated
again for 4 h at 37ºC (5% CO₂). Then, the plates were
centrifuged for 5 min at 115 x g, the supernatant was
removed, and 100 µl of dimethylsulfoxide (DMSO,
Polish Chemical Reagents S.A.) was added into each
well. After 10 min, the absorbance was read in the MRX
1.1 microreader (Dynex, U.K) at a wavelength of 620
nm.

The metabolic activity of blood phagocytes was
evaluated using the RBA (respiratory burst activity) test
after cell stimulation by PMA (Phorbol Myristate
Acetate, Sigma) described by Secombes (31), in
modification by Siwicki et al. (34). Whole blood at
amount of 100 µl was poured into the 96-well plates
(NUNC), next RPMI 1640 with 0.1% FCS (Sigma) at
amount of 100 µl/well was added into each well and
incubated for 24 h at 4°C. After incubation, the non-
adhered cells were removed by drawing off a fluid and
replacing it by the addition of 100 µl/well of 0.1% NBT
solution in RPMI 1640 or of 0.1% NBT solution in
RPMI 1640 with PMA – at amount of 1 µl/mL of 0.1%
NBT solution. The plates were incubated for 30 min at
37ºC. After incubation and the removal of the medium,
the cells were washed three times with 70% ethyl
alcohol. Cellular H₂O₂ production was measured in the
MRX 1.1 microreader (Dynex, U.K) at a wavelength of 620
nm.

The potential killing activity of blood phagocytes was
measured by means of the spectrophotometric method using the PKA (potential killing activity) test according to Rook et al. (29), in
modification by Siwicki and Anderson (32). Whole
blood at amount of 100 µl was poured into the 96-well plates
(NUNC), next RPMI 1640 with 0.1% FCS (Sigma) at
amount of 100 µl/well was added into each well and
incubated for 24 h at 4°C. After incubation, the non-adherent cells were removed by drawing off a fluid and
replacing it by 0.1% NBT solution in PBS,
containing the 18 h culture of Staphylococcus aureus
and incubated for 30 min at 37ºC. After incubation and
the removal of the medium, the cells were washed three
times with 70% ethyl alcohol. The plates were dried for
30 min and then, 120 µl of 2M KOH and 140 µl of
DMSO were added in order to dissolve formozan. The

The kinetics of variations within the range of the
potential killing activity (PKA) of blood phagocytes,
defined by its ability to intracellular killing, after the
administration of biopreparation, is presented in Table 4.

The kinetics of variations in the levels of the
proliferative response of T and B lymphocytes
stimulated by Con A and LPS mitogens during the
experiment are presented in Tables 1 and 2.

Within the scope of the proliferative response
of T lymphocytes, statistically significantly higher
response was found in group I – stimulated and
vaccinated, in comparison with groups II – stimulated,
III – vaccinated, and C - control on the 14th d after the
biopreparation administration. Statistically significantly
higher response was also observed on the 21st d in
groups I and III compared with groups II and C. On the
42nd d after the administration of the immunomodulator
and/or immunisation, a significant decrease in the
proliferative response of T lymphocytes was found in all
experimental groups, and a statistically significantly
weaker proliferative response in groups II and C in
comparison with group I.

Within the scope of the proliferative response
of B lymphocytes, on the 14th d of the experiment it was
also statistically significantly higher in group I in
comparison with groups II, III, and C, whereas on the
21st d in groups I and III compared with groups II and C. On the
42nd d after the administration of the immunomodulator
and/or immunisation, a more radical, and greater than in case of
T lymphocytes, decrease in the proliferative response of
B lymphocytes was found in all studied groups as well
as statistically significantly weaker response in group C
compared with group I.

The development of the metabolic activity of
blood phagocytes after the Bioimmuno administration
and/or immunisation with Respisure One, measured by
the respiratory burst activity level after cell stimulation
by PMA, is presented in Table 3.

On the 3rd d after the biopreparation
administration, a statistically significant increase
(P<0.05) in the metabolic activity of blood phagocytes
was found in groups I and II in comparison with group
C. On the 7th d after the immune system stimulation,
statistically significantly higher metabolic activity was
observed in all experimental groups compared with
group C, lasting until the 21st d of the experiment in
group I in comparison with groups II, III, and C, and
until the 42nd d in group I compared with group C.

The kinetics of variations within the range of the
potential killing activity (PKA) of blood phagocytes,
defined by its ability to intracellular killing, after the
administration of biopreparation, is presented in Table 4.
**Table 1**

Proliferative response of Con A-stimulated T lymphocytes after the Bioimmuno administration and/or immunisation with Respisure One

<table>
<thead>
<tr>
<th>Group</th>
<th>Extinction (620 nm)</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>I (B+R) X</td>
<td>0.17 ±0.07</td>
<td>0.11 ±0.03</td>
</tr>
<tr>
<td>II (B) X</td>
<td>0.12 ±0.03</td>
<td>0.15 ±0.07</td>
</tr>
<tr>
<td>III (R) X</td>
<td>0.12 ±0.04</td>
<td>0.15 ±0.09</td>
</tr>
<tr>
<td>C X</td>
<td>0.12 ±0.06</td>
<td>0.11 ±0.02</td>
</tr>
</tbody>
</table>

B – Bioimmuno, R – Respisure One, C – Control, ± SD.

<sup>a, b</sup> – differences between groups at P<0.05.

**Table 2**

Proliferative response of LPS-stimulated B lymphocytes after the Bioimmuno administration and/or immunisation with Respisure One

<table>
<thead>
<tr>
<th>Group</th>
<th>Extinction (620 nm)</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>I (B+R) X</td>
<td>0.15 ±0.08</td>
<td>0.11 ±0.02</td>
</tr>
<tr>
<td>II (B) X</td>
<td>0.10 ±0.02</td>
<td>0.13 ±0.06</td>
</tr>
<tr>
<td>III (R) X</td>
<td>0.11 ±0.03</td>
<td>0.13 ±0.06</td>
</tr>
<tr>
<td>C X</td>
<td>0.11 ±0.04</td>
<td>0.11 ±0.01</td>
</tr>
</tbody>
</table>

Explanations as in Table 1.

**Table 3**

Metabolic activity of PMA-stimulated blood phagocytes after the Bioimmuno administration and/or immunisation with Respisure One

<table>
<thead>
<tr>
<th>Group</th>
<th>Extinction (620 nm)</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-2</td>
<td>0</td>
</tr>
<tr>
<td>I (B+R) X</td>
<td>0.26 ±0.03</td>
<td>0.56 ±0.25</td>
</tr>
<tr>
<td>II (B) X</td>
<td>0.30 ±0.02</td>
<td>0.53 ±0.19</td>
</tr>
<tr>
<td>III (R) X</td>
<td>0.27 ±0.02</td>
<td>0.48 ±0.13</td>
</tr>
<tr>
<td>C X</td>
<td>0.29 ±0.09</td>
<td>0.49 ±0.18</td>
</tr>
</tbody>
</table>

Explanations as in Table 1.
Table 4
Potential killing activity of blood phagocytes after the administration of Bioimmuno and/or immunisation with Respisure One

<table>
<thead>
<tr>
<th>Group</th>
<th>Extinction (620 nm)</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-2</td>
</tr>
<tr>
<td>I (B+R)</td>
<td>X 0.34 ±0.01b</td>
<td>0.39 ±0.08</td>
</tr>
<tr>
<td>II (B)</td>
<td>X 0.35 ±0.05b</td>
<td>0.40 ±0.05</td>
</tr>
<tr>
<td>III (R)</td>
<td>X 0.24 ±0.04bd</td>
<td>0.44 ±0.08</td>
</tr>
<tr>
<td>C</td>
<td>X 0.31 0.06c</td>
<td>0.42 ±0.12</td>
</tr>
</tbody>
</table>

Explanations as in Table 1.

On the initial day of the experiment, a statistically significant difference was observed between groups I and II and group III as well as between groups III and C. An increase in the potential killing activity was found on the 7th d of the experiment in groups I, II, and III in comparison with group C, together with a higher killing activity of cells in group I compared with groups II and III, while on the 14th d after the biopreparation administration, in spite of a decrease in the killing activity, a statistically significant difference in the PKA level was noted in group I in comparison with groups II, III, and C. Moreover, a difference between group III and groups II and C was also noted. On the 21st d, the PKA level was still statistically significantly higher (P<0.05) in group I in comparison with groups II, III, and C, whereas on the 42nd d of the experiment, a dramatic decrease in the killing activity of blood phagocytes to the level similar in all groups was observed.

**Discussion**

Mechanisms of non-specific and specific cellular and humoral immunity, providing for the development of animal immunity and protecting against the adverse effect of a pathogen, are of decisive importance in terms of creating adequate organism response to vaccination against MPS (5, 7, 12). In our studies, we determined the proliferation activity of mitogen-stimulated lymphocytes, after the Bioimmuno administration and/or immunisation with Respisure One. In the stimulated and immunised group I, the proliferative response of T lymphocytes to Con A mitogen was statistically significantly higher (P<0.05) in comparison with the rest of the experimental groups on the 14th d of the experiment, as well as on the 21st d in group I and the immunised group III, compared with groups II (stimulated) and C (control), not receiving the vaccine. The proliferation activity of LPS-stimulated B lymphocytes was developed in a similar manner. The most intense response was observed in groups I and III, immunised and/or stimulated, on the 14th and 21st d of the experiment, although in group I the increase in the proliferation activity was maintained until the 42nd d of the experiment. A similar phenomena, concerning the increase in the proliferation activity of T and B lymphocytes, were observed in the course of natural Mhp infections as well as after the immunisation against SHV-1 infections (6, 14, 21). In a similar experimental system, Wiśniewski et al. (37), by means of administering immunostimulators – biotropin and levamisole, in pigs at the age of 3 months, 24 h before and on the 3rd and 5th d after immunisation with the Suivac A vaccine against Aujeszky’s disease, demonstrated a positive influence of their administration on the stimulation of cellular non-specific and specific immunity, with the simultaneous lack of influence on the increase in specific SN antibody titres. The studies carried out by Rumińska-Groda (30) indicated the possibility of effective modulation of immune processes in turkeys as a consequence of giving the Bioimmuno preparation in feedstuff and its effect on the course of the infections with HE virus and E. coli.

In our studies, on the 3rd d after the Bioimmuno and/or Respisure One administration, statistically significantly (P<0.05) higher metabolic activity of phagocytes isolated from the peripheral blood was shown in groups I (stimulated and immunised) and II (stimulated) in comparison with group C, whereas on the 7th d it was observed in all experimental groups compared with group C. The intensity of the immunological reactions occurring in this period maintained at the highest level in group I in comparison with the remaining groups up till the 42nd d of the experiment. Statistically significant variations were also found within the scope of the potential killing activity of phagocytes on the 7th d of the experiment in the
stimulated and/or immunised groups I, II, and III compared with group C. On the 14th and 21st d after the biopreparation administration, in spite of the decrease in the killing activity of phagocytes, merely a statistically significant difference in the PKA level was observed in group I in comparison with groups II, III, and C. The obtained results confirm the importance of blood phagocytes in playing a considerable role in the development of non-specific and specific immune response of an organism to antigen, because apart from the ability to synthesise and excrete various substances participating, among other things, in stimulation of the proliferation of lymphocytes or other antigen presenting cells, they are capable of phagocytosis, cytotoxicity, and cytolysis, too (7). In the experiment conducted by Rumilska-Groda (30) in turkeys, an increase in post-vaccinal humoral immunity against ND virus infection, as well as greater killing activity of macrophages and heterophils were demonstrated after the administration of the Bioimmuno preparation. In the in vitro studies carried out by Pappaterra et al. (24), after the application of the Inmodulen immunomodulator, the increase in the activity of mononuclear cells was observed as well. Similarly, Mikulska-Skupie (21) found the increase in the activity of blood phagocytes in pigs after immunisation with a vaccine against SHV-1 infection.

The application of specific immunoprophylaxis and immunomodulation, although it is a difficult matter, can be of great significance, especially in multi-herd breeding of pigs, where mixed infections of the respiratory system (PRDC) occur much more frequently and cause greater problems than monoaetiological diseases. Interactions among the participating microorganisms, or even the immunosuppressive properties of some of them (PRRSV, Mhp) may lead to functional disorders of the immune system, requiring the immunocorrective actions. This was confirmed by Markowska-Daniel et al. (17), who evaluated the condition of the swine immune system in the course of the respiratory system infections – singular Streptococcus suis and mixed S. suis, Mhp, and PRRSV, and found in both infections substantial differences in the T and B lymphocyte subpopulations. Although the restoration of the used populations of cells engaged in eliminating the mixed infection by the immune system was stated, their function, expressed by the ability to produce cytokines and to respond to them, was undermined.

Summarising the performed studies, it should be stated that the Bioimmuno immunomodulator and/or the Respisure One vaccine stimulate the proliferative response of T and B lymphocytes as well as they increase the metabolic activity and the potential killing activity of blood phagocytes, contributing to the improvement in the immune system functioning and to the protection of the swine organism against the respiratory system infections.

References