Arch Immunol Ther Exp, 2005, 53, 534–539
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Original Article

Received: 2005.01.26
Accepted: 2005.07.09
Published: 2005.12.15

Selective indices on non-specific cell-mediated immunity in rabbits immunized with *Chlamydophila psittaci*

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Source of support: by the State Committee for Scientific Research (KBN, Poland) grant no. 5 P06K 037 18.

Summary

Introduction: Studies of non-specific immunity in infection or immunization with *Chlamydophila* (*Chl.*) *psittaci* in static experimental models showed changes in parameters of this immunity. The aim of this study was to evaluate selected parameters of non-specific immunity in rabbits immunized with *Chl. psittaci*.

Materials and Methods: The study was performed on rabbits, divided into two groups of 10 animals each. The rabbits of the first group were immunized with *Chl. psittaci* strain 6BC and those of the second group were control animals. Blood samples were examined 9 times every 7 days. Adherence capacity and ingestion capacity of polymorphonuclear (PMN) cells (index of ingestion, percentage of ingestive cells) were determined in the blood. The cidal capacity of PMN cells was determined by evaluating the reduction of nitroblue tetrazolium test by cytochemical (spontaneous and stimulated tests) and spectrophotometric techniques. The presence of specific antibodies in the sera was estimated by the complement-fixation technique.

Results: Analysis of the results of all the studied parameters of non-specific immunity showed tendencies to decrease or increase. These differences appeared on days 7–14 and lasted to days 42–56 of the experiment. Positive titers of specific antibodies appeared on day 42 after immunization, i.e. 5 weeks after the first changes in the parameters of non-specific immunity.

Conclusion: The alterations noted in the parameters of non-specific immunity may prove useful in determining the condition of a macroorganism in contact with *Chl. psittaci*.

Key words: ***Chlamydophila psittaci*** • **rabbit** • **immune indices**

Abbreviations: **PMN** – polymorphonuclear cell (neutrophilic granulocytes), **MN** – mononuclear cell (monocyte-macrophage), **Ii** – index of ingestion, **%ic** – percentage of ingestive cells, **NBT** – nitroblue tetrazolium reduction test, **CMAC** – coefficient of metabolic activity of PMNs, **SI** – stimulation index.

Full-text PDF: http://www.aite-online/pdf/vol_53/no_6/8427.pdf

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INTRODUCTION

Chlamydophila (*Chl.*) *psittaci* belongs to the family of *Chlamydiaceae* comprising 8 serotypes, of which A, B, C, D, E, and F are pathogenic for birds and humans, horses, and turtles, while the M56 and WC serotypes induce disease in muskrats and cattle[7]. Studies on the reactivity of elements of non-specific cell-mediated immunity in humans and animals infected or immunized with microbes belonging to *Chlamydophila* sp. and *Chlamydia* sp. demonstrated that such conditions result in elevated phagocytic activity of polymorphonuclear (PMN) and mononuclear (MN) cells[2, 10, 12, 20, 28, 31, 34] and inhibit intracellular killing mechanisms of these cells through inhibition of phagosome/lysosome fusion[8, 9, 32]. Such infection was also found to augment the secretion of hydrogen peroxide (H_2O_2), superoxide anion[26, 35], lactic dehydrogenase (LDH)[33], interleukin (IL)-8, and MIP-1α[21, 22] by the cells. The released H_2O_2 may directly kill the bacteria or activate, with mediation of IL-8 or MIP-1α, other elements of the immune system, e.g. T lymphocytes[21, 22]. It should, however, be noted that studies on the infection or immunization of mammals with *Chlamydophila* sp. and *Chlamydia* sp. were most frequently concerned with determining individual indices of non-specific cell-mediated immunity. The present study aimed at evaluating alterations in the adherence capacity, ingestion capacity, and potential cidal activity of PMN cells as measured by the nitroblue tetrazolium reduction (NBT) test in rabbits, the model animal in biological studies on humans and animals immunized with *Chl. psittaci* strain 6BC (earlier termed *Chlamydia psittaci* strain 6BC). The titers of specific anti-*Chlamydia* sp. antibodies were also established in the animal sera.

MATERIALS AND METHODS

The studies were performed on 20 mixed-breed rabbits each weighing 2.5–4.0 kg, classified as animals of the CV–III group or conventional animals free of pathology[13]. The rabbits were kept in a vivarium under living and zoo-hygienic conditions consistent with national standards[13] and were fed full portions of LSK rabbit chow. The animals of group I, comprising 10 rabbits (the immunized group), received an intramuscular (i.m.) injection of *Chl. psittaci* strain 6BC (previously termed *Chlamydia psittaci* biotype 3–9) antigen, isolated in the USA from a human individual infected by a parrot, composed of 50 μg protein/ml dissolved in 1 ml sterile saline on the first and 7th days of the experiment. The animals of the control group (10 rabbits) were injected i.m. in parallel with the same amount of sterile saline. Blood was sampled on the first day, i.e. before administration of *Chl. psittaci*, from both groups of rabbits and then every 7 days (i.e. a total of 9 times in 56 days).

ESTIMATION OF NON-SPECIFIC CELL-MEDIATED IMMUNITY

The adherence capacity of peripheral blood PMN cells was determined using the method of Lorente et al.[18], based on scoring the percentage of PMN cells which adhere to glass beads of 3 mm in diameter (Sigma, USA). The ingestion capacity of PMN cells was estimated against the standard strain 209P of *Staphylococcus aureus*, as described by Brzuchowska[1] and Ładosz[19], in the modification of Deptuła[5]. Ingestion capacity was presented as the index of ingestion (Ii) denoting the mean number of bacteria ingested by a single neutrophilic granulocyte (PMN cell), scored in 100 cells exhibiting the capacity. The percentage of ingestive cells (%ic) was calculated as the proportion of PMN cells demonstrating ingestion capacity per 100 consecutive PMN cells encountered under the microscope.

Cidal activity was determined by evaluating the reduction of NBT by PMN cells employing a cytochemical technique in spontaneous and stimulated tests as well as spectrophotometry. The spontaneous and the stimulated NBT reduction tests were performed as described in the modified technique of Park et al.[23] and the spectrophotometric test according to a modified technique of Raman and Poland[25]. The coefficient of metabolic activity of PMN cells (CMAC) was also determined for the spontaneous and the stimulated NBT tests according to Grządzielska[11] as well as the stimulation index (SI) according to Lechowski et al.[17]

Serological studies

The presence and titers of anti-*Chlamydia* sp. antibodies in the rabbit sera were estimated by a complement-fixation test employing *Chlamydia* sp. antigen (Dessau, Germany).

Analysis of the results

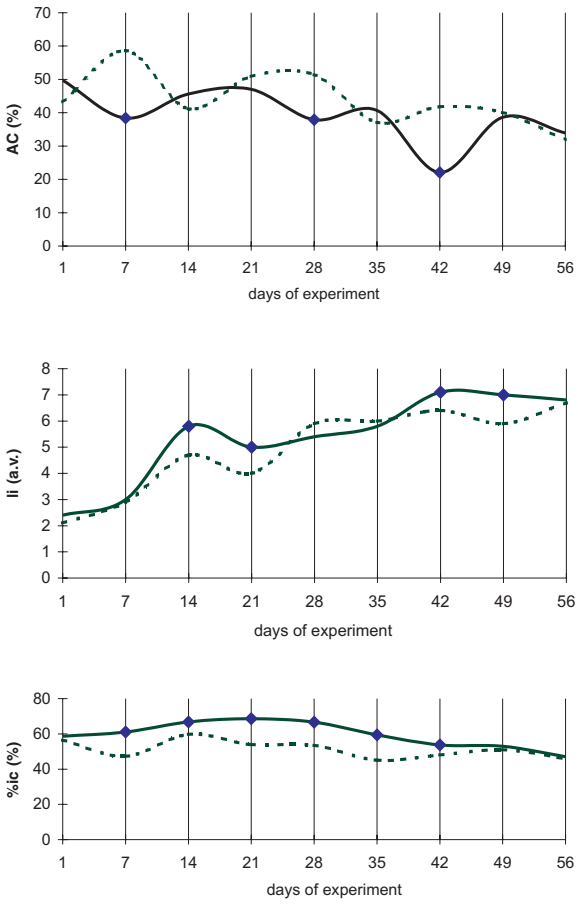
Evaluation of the results of the studies of the immune parameters is presented in Figs. 1–3 as trends of changes in their values in the immunized rabbits as well as significant differences compared with the control rabbits as evaluated by Student's *t*-test at $p \leq 0.05$.

RESULTS

Analysis of the results demonstrated that in all the studied parameters of non-specific cell-mediated

immunity in rabbits immunized with *Chl. psittaci* strain 6BC (Figs. 1–3), tendencies for a decrease or increase were noted. On the other hand, significant differences were observed in only 8 parameters, including adherence capacity (AC), Ii, %ic, the spontaneous and spectrophotometric NBT reduction tests, SI, and in values of the spontaneous and stimulated CMAC tests. No alterations were observed in the stimulated NBT reduction test.

In the analysis of AC values, a decreasing trend was observed throughout the course of the experiment (Fig. 1), but a significant decrease in the values was observed only on days 7, 28, and 42 following immunization. An increasing tendency in Ii values was observed on days 7–56 of the experiment, but the increases proved significant only on days 14, 21, 42, and 49 (Fig. 1). The %ic values also manifested an increasing tendency, persisting until day 42, and at that time the increase was significant (Fig. 1). Results of the spontaneous NBT reduction test showed variation, with a significant increase on days 14 and 42 of the study and a significant decrease on day 56 (Fig. 2). In the stimulated NBT test, only a slight increasing tendency was observed (Fig. 2), but the alterations proved insignificant. In the spectrophotometric NBT reduction test an increasing tendency was observed with certain variations, with a significant increase noted between days 35 and 56 and a significant decrease on day 7 (Fig. 2). Values of SI also demonstrated variations, with a significant increase noted on days 35 and 56 and a significant decrease on days 14, 21, and 42 (Fig. 3). Results of the spontaneous CMAC test showed a decreasing tendency with variation, with significant increases noted on days 14 and 28 and significant decreases on days 7, 21, 35, 42, 49, and 56 (Fig. 3). Results of the stimulated CMAC test showed a decreasing tendency only, and significant decreases were observed on days 7, 14, 28, 42, 49, and 56 (Fig. 3).



Explanations:
animals immunized with *Chlamydophila psittaci* – strain 6BC;
◆ significant differences between immunized and control animals;
control animals;
a.v. – absolute value.

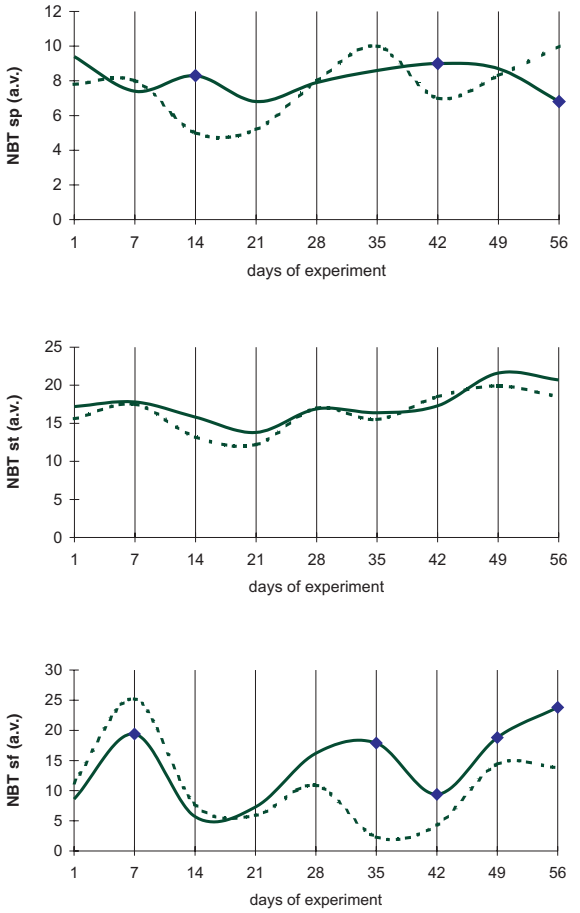
Figure 1. Adherence capacity (AC), index of ingestion (Ii) and percentage of ingestive cells (%ic) in rabbits immunized with *Chl. psittaci* – strain 6BC and in control rabbits.

Results of the serological studies showed that specific anti-*Chlamydia* sp. antibodies started to appear on the 7th day, but positive titers of the antibodies (positive titer according to the instruction[14], i.e. a 4+ titer in dilutions of 1:16), did not develop until the 42nd day of the study.

DISCUSSION

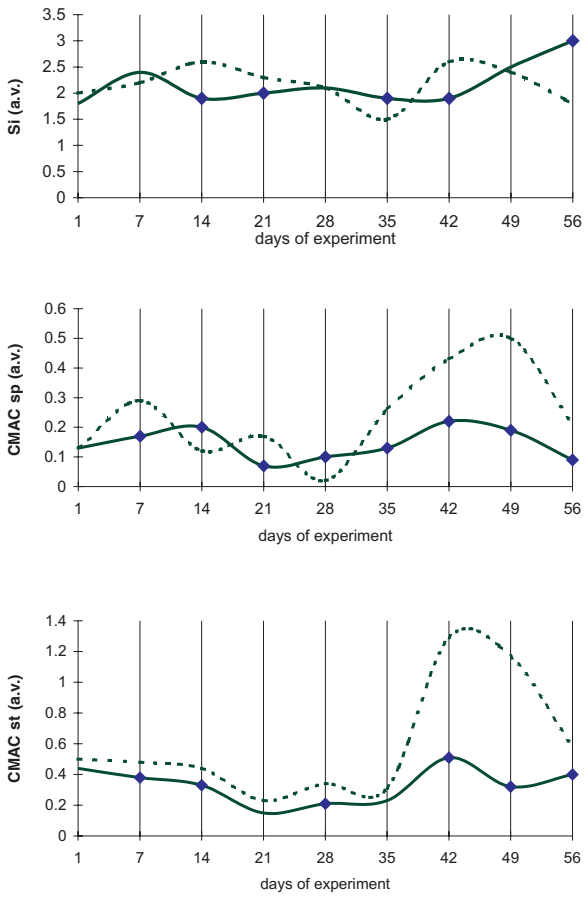
In analyzing these results it should be noted that the detected decrease in adherence capacity in rabbits immunized with *Chl. psittaci* strain 6BC developing on days 7, 28, and 42 contrasted with our earlier results obtained in rabbits immunized with *Chl. psittaci* strain 6BC[24]. In the studies[24] on rabbits immunized with an analogous antigen, an augmented adherence was observed between days 7 and 56 after immunization. A similar increase in adherence capacity was noted in pigs experimentally infected with the intracellular bacteria *Salmonella choleraesuis*[29], in which the increase developed between 6 and 48 h after infection. The data indicate that the recorded decreased or augmented adherence of PMN cells following stimulation with *Chlamydia psittaci* prove that this trait of the phagocytotic process changes in the course of immunization with intracellularly parasitizing bacteria. This hypothesis was also confirmed by results obtained in animals infected with *Salmonella* sp.[30] which, similarly to *Chlamydia* sp., resides intracellularly.

On the other hand, the observed increase in the Ii of PMN cells on days 14, 21, 42, and 49 and the increase



Explanations:
animals immunized with *Chlamydophila psittaci* – strain 6BC;
◆ significant differences between immunized and control animals;
control animals;
a.v. – absolute value.

Figure 2. NBT reduction test (spontaneous – sp, stimulated – st, and spectrophotometric – sf) in rabbits immunized with *Chl. psittaci* strain 6BC and in control rabbits.



Explanations:
animals immunized with *Chlamydophila psittaci* – strain 6BC;
◆ significant differences between immunized and control animals;
control animals;
a.v. – absolute value.

Figure 3. Stimulation index (SI) and coefficient of metabolic activity of PMN cells (CMAC) for the spontaneous (sp) and stimulated (st) NBT reduction test in rabbits immunized with *Chl. psittaci* – strain 6BC and in control rabbits.

in %ic on days 7, 14, 21, 28, 35, and 42 remain inconsistent with our own earlier results[24] obtained in rabbits immunized with an analogous bacteria, where no alterations were noted. However, it should be added that the present results corroborate observations of other authors[2, 8–10, 12, 20, 31, 34] who found that infection with *Chl. psittaci* strain 6BC results in an augmented ingestion capacity of PMN or MN cells both *in vivo* and *in vitro*. A similar increase in ingestion capacity was observed in peritoneal macrophages in mice experimentally infected with *Chl. psittaci* strain 6BC[12]. Hence it may be assumed that the results obtained in the present study concerning ingestion capacity confirm the notion that the bacteria increase the ingestion ability of PMN and MN cells. The notion was also confirmed by results on the ingestion capacity of PMN in pigs experimentally infected with *Salmonella choleraesuis*[29], in which the capacity increased already 48 h following inoculation of the animals with the microbes. In cases of experimental infection with other intracellularly residing bacteria, such as *Salmonella typhimurium*[15], *Listeria monocytogenes*[4], or *Francisella tularensis*[28], spleen and liver PMN cells demonstrated, in turn, lowered ingestion activity. Our results on the ingestion capacity of PMN and MN cells find confirmation in studies demonstrating that *Chlamydia* sp. and *Chlamydophila* sp. affect the ingestion ability of PMN and MN cells[2, 8–10, 12, 20–22, 24, 31, 32] and of cells of the L and HeLa cell lines[3]. Thus it should be concluded that the observed alterations in ingestion capacity of PMN and MN cells in the course of interaction of *Chlamydia* sp. or *Chlamydophila* sp. with the macroorganism are specific and represent an important protective mechanism in the infections.

The increase detected in the present study (on days 14 and 42) in the values of the spontaneous NBT test,

paralleled by unchanged values of the stimulated NBT test, remains difficult to interpret due to the lack of analogous studies. On the other hand, the increase we detected in the values of the spectrophotometric NBT test between days 35 and 56, preceded by a decrease on the 7th day, is consistent only when the decrease is considered with the results obtained in rabbits immunized with *Chl. psittaci* strain 6BC[6]. Similarly, the changes observed in the present study in the values of the spontaneous and the stimulated CMAC and in the stimulation index remain difficult to interpret due to the absence of analogous studies in the literature. As far as the cidal activity of PMN cells is concerned, measured by the spontaneous and the spectrophotometric NBT reduction tests in rabbits immunized with *Chl. psittaci*, the values of which tended to increase, the results are consistent with those of other authors[26, 33, 35]. The authors found in *in vitro* culture of PMN cells[33] and MN cells[26, 35] that infection with *Chlamydia psittaci* strains 6BC and Cal 10[26, 33], as well as with *Chlamydia trachomatis* serotype L_2[35], induced an increase in the cidal activity of the cells. They also demonstrated that the increase was related to the augmented activity of superoxide anion and H_2O_2[26, 35] as well as LDH[33]. In other studies[21, 22], infection with *Chl. abortus* in mice was found to induce augmented secretion also of IL-8 and MIP-1α by PMN cells. The notion of elevated activity of PMN and MN cells due to their interaction with intracellularly residing bacteria has been confirmed by studies performed on mice infected with bacteria which also reside intracellularly, such as *Mycobacterium tuberculosis*[15]. Results on the cidal activity of PMN cells obtained by other authors[6, 15, 21, 22, 26, 33, 35] as well as the results of our own studies prove that changes in cidal activity resulting from PMN reaction with such bacteria reflect alterations in both the oxygen-dependent cidal system and the oxygen-independent cidal system, related to hydrolytic enzymes. This is confirmed by the elevated cidal activity of PMN and MN cells in mice experimentally infected with intracellular parasites such as *Listeria* sp. or *Rickettsia* sp.[15], in which augmented activity of tumor necrosis factor α was demonstrated.

Positive titers of specific anti-*Chlamydia* antibodies, demonstrated in the studied rabbits starting on the 42nd day after immunization, confirmed earlier data[24] in which the appearance of specific anti-*Chlamydia* antibodies was documented in the 5th week following immunization of rabbits with *Chl. psittaci*. It should be added that similar results have been obtained in humans naturally infected with *Chl. psittaci*[16], in whom positive titers of the antibodies were recorded 50 days after detection of clinical signs/symptoms, and in sheep[27], in which the titers appeared 34–54 days after administration of anti-chlamydial vaccine.

In conclusion, immunization of rabbits with *Chl. psittaci* strain 6BC induced increases as well as decreases in the values of 8 examined indices of nonspecific cell-mediated immunity, except for the stimulated NBT reduction test. The alterations developed between days 7 and 14 of the studies and persisted until days 42–56. The pattern of alterations, noted 5 weeks earlier than the appearance of positive titers of specific anti-*Chlamydia* antibodies, suggests that the studied indices may prove useful in defining the condition of an organism in the course of contact with *Chl. psittaci* bacteria.

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