Dynamics of lymphocytes T and B and of their subpopulations in peripheral blood of rabbits immunized with *Chlamydophila psittaci*

MAŁGORZATA PAWLIKOWSKA, WIESŁAW DEPTUŁA

Chair of Microbiology and Immunology, Faculty of Natural Sciences, University of Szczecin, Poland

Abstract

The studies aimed at presenting dynamic alterations in levels of lymphocytes T (receptor CD5+), Th (receptor CD4+), Tc/Ts (receptor CD8+), B (receptor IgM) and of lymphocytes with CD25+ receptor in rabbits immunized with Chlamydophila psittaci *– strain 6BC. Serological studies were also conducted to detect presence of specific anti-*Chlamydophila *antibodies. Analysis of the results demonstrated that immunization of rabbits with the studied antigen resulted in an increase in levels of lymphocytes B in days 14, 28, 42, 49, 56, lymphocytes with CD25 receptor in days 14, 42, 49, lymphocytes T in days 14 and 35, Tc/Ts cells in days 28 and 35 and decreased levels of lymphocytes Th in days 7, 14 and 56, lymphocytes T in days 7, 42 and 49, lymphocytes Tc/Ts in the day 42 and lymphocytes with CD25 receptor in the day 21. On the other hand, positive titres of specific antibodies were detected in days 49-56 following immunization, i.e. 5-6 weeks later than the first significant alterations in levels of lymphocytes and of their subpopulations.*

Key words: *lymphocytes T, lymphocytes B,* Chl. psittaci*, dynamics, immunization.*

(Centr Eur J Immunol 2007; 32 (3): 164-168)

Introduction

The genus of *Chlamydophila* (*Chl.*) *sp.*, together with the *Chlamydia sp.* genus forms the *Chlamydiaceae* family of the *Chlamydiales* order [1-3]. The genus comprises six species: *Chl. psittaci*, *Chl. abortus*, *Chl. felis*, *Chl. caviae*, *Chl. pecorum* and *Chl. pneumoniae* [1-3]. Depending on their biotype and serotype, the bacteria induce various diseases in humans and in animals (table 1).

Studies on resistance phenomena in infections or following immunizations of animals and humans with bacteria of *Chlamydophila sp.* and *Chlamydia sp.* genera demonstrated that even if the immunity linked to lymphocytes and their subpopulations plays a significant role, few respective observations are available and the relevant studies were performed in static systems. For example, in mice experimentally infected with *Ch. psittaci* – strains Ab7 and 1B or with *Ch. pecorum* – strain iB1 [6] increases in lymphocyte T (receptor CD2), Th (receptor CD4) and Tc/Ts (receptor CD8) levels were observed. An analogous increase in the level of B cells was noted in mice experimentally infected with *Ch. trachomatis* – biotypes LGV and trachoma or *Ch. psittaci* – strain MnPn [7]. Such a pattern, i.e. increased levels of lymphocytes Th and B was noted also in turkeys following immunization with MOMP protein of *Chl. psittaci* – strain 84/55 (serotype D) [8]. On the other hand, natural infection with *Ch. psittaci* in humans [9] permitted to find out that the infection was not always followed by changes in the pool of lymphocytes T, but in vitro was found to affect their transformation and proliferation under effect of concavalin A, pokeweed mitogen or phytohaemagglutinin. In cattle, natural infection with *Chl. psittaci* [10] was followed by augmented levels of lymphocytes B and lymphocytes T (receptor CD2), Th (receptor CD4) and Tc/Ts (receptor CD8).

Correspondence: Małgorzata Pawlikowska, Chair of Microbiology and Immunology, Faculty of Natural Sciences, University of Szczecin, Fe□

Table 1. Characteristics of bacteria of *Chlamydophila sp.* order [1, 2, 4]

Species	Biotype	Serotype	Pathology
*Chlamydophila psittaci**	lack of data	A, B, C, D, E, F, M56	*Psittacosis* of wild and household birds, abortions, pneumoniae in humans
		WC	enteritis in cattle
*Chlamydophila abortus**	lack of data	lack of data	abortions, weak newborns in ruminants (cattle, goats, sheep), abortions in horses, rabbits, guinea pigs, mice, pigs and in women
Chlamydophila felis	lack of data	probably 4 (?)	conjunctivitis, nasal mucositis in cats
Chlamydophila caviae	lack of data	lack of data	conjunctivitis, mucositis, infections of genital tract in guinea pigs
Chlamydophila pecorum	lack of data	lack of data	pathology of genital and urinary systems (koala); abortions, conjunctivitis, enteritis, pneumoniae, meningitis, polyarthritis (ruminants)
Chlamydophila pneumoniae	TWAR	TW-183, AR-37, AR--277, AR-388, AR-427, AR-231, LR-65	acute or chronic bronchitis and pneumoniae, potential for arteriosclerosis, myocardial infarction, coronary and ischemic disease of the heart, Reiter's syndrome and sarcoidosis, Alzheimer disease, multiple sclerosis in humans
	Koala	lack of data	infections of respiratory tract in koala
	Equine	lack of data	pathology of respiratory tract in horses

* – *The* □

It should be added that in other studies employing various experimental models (humans, mice, sheep, cattle, turkeys, cell lines) infection with *Chlamydia sp.* or *Chlamydophila sp.* Was found to increase levels of lymphocytes T [11-14] and lymphocytes B [6, 13, 15, 16].

Summing up the till now registered observations it should be noted that demonstration of involvement of lymphocytes T and B as well as of their subpopulations in animals and humans infected with or immunized with *Chlamydia sp.* or *Chlamydophila sp.* bacteria, has not been followed by quantitative studies on the cells. Therefore, our studies aimed at determining numbers of lymphocytes T, Th, Tc, B and lymphocytes with CD25 receptor in the post-immunization period in peripheral blood of rabbits - the model animal for human and animal pathology, immunized with the ubiquitous strain of *Chlamydophila psittaci*.

Material and Methods

The studies were performed on 20 rabbits of mixed breed, in 4 groups of 10 animals each, weighing each 2.5 kg to 4.0 kg, classified as conventional animals [17]. In course of the studies the animals were housed in a vivarium of the Chair of Microbiology and Immunology, in which zoohygienic and housing criteria corresponded to legal standards binding in the country [18].

In two groups of immunized rabbits every animal received in the first and the seventh days of the experiment, into the rear extremity, intramuscular injection of *Chlamydophila psittaci* – strain 6BC antigen, isolated from a man infected by a parrot, dissolved in 1 ml sterile physiological saline to protein concentration of 50 μg/ml. Animals of the control group received at the same time and in the same amount intramuscular injection of a sterile physiological saline.

Blood for tests was sampled to sodium versenate through a venflon from a marginal ear vein in the first day, i.e. before administration of *Chl. psittaci* in the groups of experimental rabbits or before administration of physiological saline in the group of control animals. Subsequently, blood tests in the experimental rabbits were conducted on 8 occasions, spaced by 7 days, i.e. on days 7, 14, 21, 28, 35, 42, 49 and 56 following the immunization.

Determination of lymphocytes T and B and of their subpopulations

The determination took advantage of a flow cytometer (Cytoron Absolute, Ortho, USA) according to the technique described by Deptuła et al. [19]. The technique used for detection of lymphocytes and their subpopulations monoclonal antibodies (mouse anti-rabbit antibodies; Serotec, USA) capable of identifying lymphocytes B with IgM receptor, lymphocytes T carrying CD5+ receptor, lymphocytes Th with CD4+ receptor, lymphocytes Tc/Ts with CD8+ receptor and lymphocytes carrying CD25+ receptor.

Serological studies

Presence and titre of antibodies to *Chlamydophila sp.* In rabbit sera were determined by complement fixation test, executed according to manufacturer's instruction [20], in which a positive titre is denoted by inhibition of hemolysis of ++ intensity in serum dilutions of 1:32 and higher.

Statistical analysis

Results of the immune tests were subjected to statistical analysis using Student's *t*-test at p=0.05, comparing the obtained results with those found in rabbits of control gro-

ups, and were presented in forms of arithmetic mean and standard deviations (SD+) in table 2. Serological results are shown in table 3.

Results

Analysis of results showed that *Chlamydophila psittaci* – strain 6BC induced a statistically significant increase only in levels of lymphocytes B. The levels of lymphocytes with CD25 receptor and of lymphocytes Tc/Ts and T manifested an increase and a decrease, while levels of lymphocytes Th manifested a significant decrease (table 2). Increase in levels of lymphocytes was observed in days 14, 28, 42, 49 and 56 of the experiment while increase in lymphocytes with CD25 receptor was noted in days 14, 42 and 49, increase in lymphocytes T was seen in days 14 and 35, and increase in lymphocytes Tc/Ts was detected only in days 28 and 35. Decreased levels of lymphocytes Th were recorded in days 7, 14 and 56, decreased levels of lymphocytes T in days 7, 42 and 49, decreased levels of lymphocytes Tc/Ts in day 42 and decreased levels of lymphocytes with CD25 receptor in day 21 of the experiment.

Table 2. Numbers of lymphocytes B and T and of their subpopulations in peripheral blood of rabbits immunized with *Chlamydophila psittaci* – strain 6BC ($\bar{\chi}$ and SD±)

Lymphocytes with a specific CD receptor (%)	Numbers in individual days																	
	1↓		7↓		14		21		28		35		42		49		56	
	Z	K	Z	K	Z	K	Z	K	Z	K	Z	K	Z	K	Z	K	Z	K
lymphocytes B (IgM)	16.3	8.8	12.6	8.4	14.1*	4.8	9.6	8.6	12.9*	5.0	10.9	12.6	20.5*	16.5	24.8*	18.6	20.2*	16.1
	3.5	2.1	4.2	4.3	3.4	1.5	3.4	3.0	2.9	2.2	3.1	1.5	3.3	3.5	1.6	1.4	3.7	2.2
lymphocytes T ($CD5^+$)	55.9	52.3	47.1	60.4*	50.5*	42.4	57.0	52.0	58.0	55.4	58.5*	44.3	34.3	48.5*	47.9	53.1*	54.4	55.2
	7.1	8.7	3.9	3.6	9.1	8.0	10.0	13.8	10.7	13.9	6.9	6.6	10.3	10.5	5.6	6.6	10.0	9.5
lymphocytes Th ($CD4^+$)	34.2	32.6	37.4	44.5*	34.2	50.2*	35.4	38.8	34.4	32.3	35.8	32.9	31.8	32.4	36.6	33.9	37.1	43.5*
	8.6	7.8	6.6	7.7	8.5	8.6	7.4	9.0	6.6	6.7	10.9	11.1	7.0	7.3	7.8	8.0	8.9	8.2
lymphocytes Tc/Ts ($CD8^+$)	16.4	17.6	14.9	13.7	14.9	12.5	15.7	13.8	17.5*	14.5	23.3*	15.1	13.8	22.1*	11.4	14.1	14.2	14.5
	3.3	2.9	3.6	3.7	3.0	3.5	5.2	4.7	2.1	1.2	8.5	7.0	3.7	2.7	2.4	3.6	1.1	2.2
lymphocytes $CD25^+$	7.3	6.5	7.4	7.6	5.6*	3.0	2.2	4.3*	3.7	3.1	4.3	3.4	10.5*	7.6	12.0*	8.7	8.7	8.8
	2.6	2.9	3.8	4.4	1.4	1.4	0.5	0.7	1.3	1.4	1.3	1.5	2.6	2.0	2.6	2.7	1.5	2.1

*Z – immunized animals; K – control animals; ↓ – day of immunization; * – difference significant at p≤0.05.*

Table 3. Results of serological studies in rabbits immunized with *Chl. psittaci*

Day of studies	Chlamydophila psittaci – strain 6BC dilution of serum					
	1:2	1:4	1:8	1:16	1:32	1:64
1	--	--	--	--	--	--
7	--	++++	+	--	--	--
14	++++	++++	++	+	--	--
21	++++	++++	+	--	--	--
28	++++	++++	++	+	+	--
35	++++	++++	++++	+++	+	--
42	++++	++++	++++	++++	+	--
49	++++	++++	++++	++++	++	++
56	++++	++++	++++	++++	++	+

Inhibition of hemolysis deter□
marked by 2 pluses (++) or more pluses at the dilution of 1:32 was regarded to represent a positive titre [20].

Analysis of results obtained in serological studies (table 3) demonstrated that positive titres obtained according to the binding instruction [20] did not appear in rabbits immunized with *Chl. psittaci* – strain 6BC until days 49 and 56 following the immunization.

Discussion

In present studies the increase in peripheral blood lymphocytes B observed in rabbits immunized with *Chl. psittaci* confirmed observations of Levitt et al. [7], who demonstrated proliferation of mouse lymphocytes B *in vitro* following their stimulation with *Ch. psittaci* – strain MnPn and *Ch. trachomatis* – biotype LGV. On the other hand, distinct results in the number of lymphocytes B were obtained by Buendia et al. [6], who observed that infection in mice with *Ch. psittaci* – strain AB7 and 1B and with *Ch. pecorum* – strain iB1, induced in their spleens a decreased number of the cells. Still another pattern of lymphocytes B in the genital tract was obtained by Maxion et al. [13] in mice experimentally infected with *Ch. muridarum*. Also in the cattle naturally infected with *Chl. psittaci* [10] and in turkeys immunized with a plasmid coding MOMP proteins of *Chl. psittaci* [8] augmented peripheral blood levels of lymphocytes B were noted. Involvement of lymphocytes B in anti-chlamydial immunity was confirmed also by Morrison et al. [15] who studied effects of re-infection with *Ch. trachomatis* in mice with depleted lymphocytes B and demonstrated that animals with depleted lymphocytes B were particularly prone to another infection with the bacteria. Moreover, the team of Ramsey [16] demonstrated in mice that augmented activity of lymphocytes B due to their infection with *Ch. trachomatis* induced a compensatory response of other specific mechanisms: the delayed type response conditioned by lymphocytes T.

Determination of numbers of lymphocytes T, obtained in a dynamic system in rabbits immunized with *Chl. psittaci*, demonstrated that their alterations manifest by both augmented and, in other time points, by decreased levels of lymphocytes T, Tc/Ts and of lymphocytes with CD25+ receptor but only by a decrease in level of lymphocytes Th, which pointed to the role of lymphocytes T and their subpopulations in anti-chlamydial immunity. The results have confirmed the data of till now performed studies [6, 8-14, 21, 22], which, however, were examining single time points, i.e., were not performed in a dynamic manner. Comparison of alterations in numbers of lymphocytes T and of their subpopulations in rabbits immunized with *Chl. psittaci* has found partial confirmation in studies of Buendia et al. [6], who in spleens of mice infected with *Chlamydophila psittaci* recorded not only increased numbers of lymphocytes T with CD4 receptor but also a decrease in lymphocytes T with CD8 receptor. In cattle naturally infected with *Chl. psittaci* also increased levels of only peripheral blood lymphocytes T, Th and Tc/Ts were detected [10]. Similar results were described also in blood of turkeys immunized with a plasmid coding MOMP protein of *Chl. psittaci* – strain 84/55 (serotype D), which manifested augmented activity of lymphocytes Th [8], and in humans naturally infected with *Ch. psittaci* [9]. Also the increase in lymphocytes Th and decreased level of lymphocytes Tc/Ts recorded in spleens of mice experimentally infected with *Chlamydophila pecorum* [6] as well as decreased numbers of lymphocytes Th detected in genital tract on mice infected with *Ch. muridarum* [13] partially confirmed the presented results of our own studies. A pattern similar to that described in [13] was registered in mice experimentally infected with the germ [14], in which spleens contained elevated levels of lymphocytes T and lymphocytes Th in particular. Also in cultures of line L cells [11] and in cultures of human lymphocytes isolated from patients naturally infected with *Chlamydia trachomatis* [12] augmented levels of lymphocyte Tc/Ts activity were seen [11, 12]. The results [6, 8, 9, 11-14] were confirmed also in humans naturally infected with *Chl. pneumoniae* [21] and in mice experimentally infected with the bacteria [22], in whom/in which intensified in vitro proliferation of lymphocytes T [21] was accompanied by increased activity of mainly lymphocytes Tc/Ts originating from lungs [22]. It should be added that, despite similarities, the results of studies [6, 8-14, 21, 22] cannot unequivocally be related to results of our present work since alterations in levels of immunocompetent cells, including lymphocytes T and their subpopulations, remain slightly different in peripheral blood, as compared to spleen, liver or placenta in mammals or birds.

Nevertheless, the presented observations of our own or of other authors [6-12, 14-16, 21, 22] indicate that already due to alterations in levels of lymphocytes T and B detected in a static set-up, lymphocytes B, T, and their subpopulations represent a significant element of protection against infections with *Chlamydia sp.* and *Chlamydophila sp.* bacteria. The studies have shown that lymphocytes B, lymphocytes Tc/Ts and lymphocytes Th represent the most important cells involved in anti-chlamydial response in humans and in animals (laboratory and farm animals) due the timing of alterations in their levels. Present studies have shown that immunization of rabbits with *Chl. psittaci* induces increase in lymphocytes B only, an increase and a decrease in levels of lymphocytes with CD25 receptor, lymphocytes T and Tc/Ts and a decrease only in lymphocytes Th. The increased levels have been observed in days 7, 14, 28, 35, 42, 49 and 56 following immunization while decreased levels have been detected in days 14, 21, 42, 49 and 56 after immunization. The alterations have persisted for the longest time in levels of lymphocytes B and T. It can be concluded that the timing of increased or decreased numbers of lymphocytes B and T and their subpopulations has not been described in the till now performed studies, which were conducted in static experimental set-up and which therefore did not document time progress of the alterations. The documented

time course of the alterations represents, therefore, a new element of our knowledge on anti-chlamydial immunity.

References

1. Deptula W, Pawlikowska M, Travnicek M (2002): New data on systematics of Chlamydia (in Polish). Post Mikrobiol 41: 71-83.
2. Everett KD, Bush RM, Andersen AA (1999): Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. Int J Syst Bacteriol 49: 415-440.
3. Garrity GM: Bergey's Manual of Systematic Bacteriology. Second Edition. Volume 1. The Archaea and the deeply branching and phototropic bacteria. Ed. D. Boone, R.W. Castenholz. Springer-Verlag. New York, USA, 2001.
4. Deptula W, Pawlikowska M, Travnicek M (2002): Chlamydofiloses in animals and humans (in Polish). Medycyna Wet 58: 337-340.
5. Pawlikowska M: Alterations in selected parameters of immunity in rabbits immunized with various strains of Chlamydia sp. Doctoral thesis, Faculty of Natural Sciences, University of Szczecin. Szczecin, Poland, 2003.
6. Buendia AJ, Sanchez J, Del Rio L et al. (1999): Differences in the immune response against ruminant chlamydial strains in a murine model. Vet Res 30: 495-507.
7. Levitt D, Danen R, Bard J (1986): Both species of Chlamydia and two biovars of Chlamydia trachomatis stimulate mouse B lymphocytes. J Immunol 136: 4249-4254.
8. Van Loock M, Lambin S, Volckaert G et al. (2004): Influence of maternal antibodies on Chlamydophila psittaci-specific immune responses in turkeys elicited by naked DNA. Vaccine 22: 1616-1623.
9. Konopka L, Koba S, Partyka M et al. (1984): Disturbances of cell-mediated immunity in ornithosis. Arch Immunol Ther Exp (Warsz) 32: 177-184.
10. Niemczuk K, Bednarek D (2003): Changes in the peripheral leukocyte phenotype of calves in clinical cases of bronchopneumonia complicated with chlamydial co-infectious agent. Pol J Vet Sci 6: 125-129.
11. Beatty PR, Stephens RS (1994): CD8+ T lymphocyte-mediated lysis of Chlamydia-infected L cells using an endogenous antigen pathway. J Immunol 153: 4588-4595.
12. Goodal JC, Yeo G, Huang M et al. (2001): Identification of Chlamydia trachomatis antigens recognized by human CD4+ T lymphocytes by screening an expression library. Eur J Immunol 31: 1513-1522.
13. Maxion HK, Liu W, Chang M-H, Kelly K (2004): The infecting dose of Chlamydia muridarum modulates the innate immune response and ascending infection. Infect Immun 72: 6330-6340.
14. Shaw J, Grund V, Durling L et al. (2002): Dendritic cells pulsed with a recombinant chlamydial major outer membrane protein antigen elicit a CD4(+) type 2 rather than type 1 immune response that is not protective. Infect Immun 70: 1097-1105.
15. Morrison SG, Su H, Caldwell HD, Morrison RP (2000): Immunity to murine Chlamydia trachomatis genital tract reinfection involves B cells and CD4(+) T cells but not CD8(+) T cells. Infect Immun 68: 6979-6987.
16. Ramsey KH, Soderberg LSF, Rank RG (1988): Resolution of chlamydial genital infection in B-cell deficient mice and immunity to reinfection. Infect Immun 56: 1320-1325.
17. Anon.: Information cards for technological foundations of animal production: Laboratory animals (in Polish). In: Informative/instruction materials of the Section for Laboratory Animals, ZG SITR. Warszawa, Poland, 1987; 2: 26-77.
18. Instruction of the Principal Veterinary Doctor No. GIWzVIII/400/lab-50/2004 of 25th November, 2004, pertaining serological diagnosis of cattle chlamydiosis and endozootic abortions in sheep using a micromethod of complement fixation with elements of appraising the risk of the test (in Polish).
19. Deptula W, Kostrzewa A, Stosik M et al. (1998): Subpopulations of peripheral blood lymphocytes in rabbits. Nowiny Lekarskie 67: 377-382.
20. Instruction of the Minister of Agriculture and Country Development of 24th February, 2005 on detailed conditions of animal up-keep in cultures of laboratory animals and in institutions conducting tests or observations (Dz.U. Nr 39, poz. 374).
21. Boer de OJ, van der Wal AC, Houtkamp MA et al. (2000): Unstable atheroslcerotic plaques contain T-cells that respond to Chlamydia pneumoniae. Cardiovascular Res 48: 402-408.
22. Wizel B, Starcher BC, Samten B et al. (2002): Multiple Chlamydia pneumoniae antigens prime CD8+ Tc1 responses that inhibit intracellular of this vacuolar pathogen. J Immunol 169: 2524-2535.