Immunological response and hematological picture in rabbits immunized with *Chlamydia trachomatis*

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Abstract

The paper presents data on adherence capacity, phagocytic ability, potential cidal capacity of PMN cells in NBT reduction test, activity of myeloperoxidase in the cells, amount and activity of lysozyme in serum, total level of immunoglobulins, including IgG in serum in rabbits immunized with Chlamydia trachomatis, the bacteria which continues to pose a significant threat to humans. Levels of hemoglobin, erythrocytes and leukocytes, blood smears and presence of specific anti-Ch. trachomatis antibodies were also tested. Analysis of the immune variables indicates that immunization with the bacteria has induced alterations in just 7 out of 14 examined immune variables and that in most cases the alterations have involved decreased values and in only few cases elevated values of the parameters. Out of the 8 examined hematological parameters, alterations were noted in only three variables, with augmented and lowered levels noted with a similar frequency. The alterations, particularly those in immunological parameters, were noted 3 weeks before the appearance of specific anti-Ch. trachomatis antibodies.

Key words: *Chlamydia trachomatis, rabbit, immune indices, hematological indices.*

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Introduction

Chlamydia trachomatis represents an intracellular, Gram-negative bacterium, which is pathogenic mainly for humans and belongs to *Chlamydia* genus, *Chlamydiaceae* family. *Chlamydia trachomatis* encompasses two biotypes: *trachoma* and *lymphogranuloma venereum* (LGV). In the trachoma biotype 14 serotypes were distinguished, denoted by the letters A-K and acronyms Ba, Da, Ia, while the LGV biotype was found to contain 4 serotypes, L_1, L_2, L_{2a} and L_3. Serotypes A, B, Ba, C are linked to endemic trachoma, D, Da, E, F, G, H, I, Ia, J, K with diseases of sexual tract, conjunctivitis, pneumonia, pharyngitis, inflammation of nasal mucosa, otitis, sudden infant death syndrome (SIDS), urethritis, prostatitis and epididymitis in men, cervicitis and pelvic inflammatory disease in women, infertility women and men, stillborn fetus; while serotypes L_1, L_2, L_{2a} and L_3 are related to diseases of sexual tract, including venereal lymphogranuloma, proctitis, ulcers of genital organs and to infection in the lymphoid tissue [1 and cit. 2]. The microbe was also noted without clinical signs in cattle [3] and in swine* [4]. Even if *Chlamydia trachomatis* causes multiple commonly developing human diseases, including those of a grave course, as well as asymptomatic infections in the cattle, few studies have been devoted to immune phenomena in dynamic system in such infections in humans or animals or following immunization of humans or animals with the microbes [cit. 2].

The aim of study was top record of dynamics of chosen parameters of innate immunity and selected hematological parameters in rabbits immunized with *Ch. trachomatis*, which could possibly be related to alterations not described noted in humans following infection with the bacteria.

Materials and Methods

The studies were performed on rabbits of mixed breed, which originated from farm under veterinary and zootechnical

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surveillance [5]. The animals were kept in typical rabbit cages in the animal house, in zoo hygienic conditions corresponding to the currently binding standards. They were fed full-portion LSK chow for rabbits produced by Factory of Concentrated and Mixture Fodders 'Agropol' in Motycz, 0.15 to 0.2 kg/day with free access to tap water. The animals formed two groups, adequately 20 and 10 rabbits. Group I included immunized rabbits, in the first and the seventh days injected into the muscles of a rear extremity with *Ch. trachomatis*, biotype *trachoma*, German isolate originating from a *non--gonococcal urethritis* patient and suspended in 1 ml sterile saline and containing 25 µg protein/ml. Group II included control animals which received intramuscular injection of 1 ml sterile saline.

Blood for the tests was sampled from immunized and control animals in the first day, before injection of *Ch. trachomatis* and of sterile saline, and then on days 7, 14, 21, 28, 35, 42, 49 and 56 of the experiment.

Immunological tests

Adherence capacity of PMN cells (AC) from peripheral blood was established using the technique of Lorente et al. [cit. 2], based on the calculation of the percentage of PMN cells that exhibited adherence to a 3 mm diameter glass pearls (Sigma, USA). Ingestion capacity of PMN cells was tested with the standard strain 209P of *Staphylococcus aureus*, employing the technique of Brzuchowska [cit. 2] and Ładosz [cit. 2], in modification of Deptuła [cit. 2]. It was expressed by index of ingestion (Ii) and percentage of ingestion cells (%ic). Reduction of nitrotetrazolium blue (NBT) in neutrophilic granulocytes was determined by cytochemistry in the spontaneous and the stimulated tests, according to the modified technique of Park et al. [cit. 2] and by a modified spectrophotometric technique of Raman and Poland [cit. 2]. Coefficient of metabolic activity of the cells (CMAC) was also determined for the spontaneous and the stimulated NBT tests, according to Grządzielska [cit. 2], and the stimulation index (SI) according to Lechowski [cit. 2]. Activity of myeloperoxidase (MPO) in PMN cells was determined using the technique of Graham, as described by Zawistowski [cit. 2], while amount of serum lysozyme (LZM) was determined by the plate diffusion technique against *Micrococcus (M.) luteus* (previously *M. lysodeicticus*) strain, performed as described by Hankiewicz [cit. 2] and acitivity of LZM determined by formula described by Szmigielski [cit. 2]. Total amount of serum immunoglobulins (Ig) was evaluated according to McEwan [cit. 2], while amount of serum IgG was established by the plate diffusion technique using standards of ICN company (USA).

Hematological and serological tests

Concentration of hemoglobin, content of erythrocytes, leukocytes, white blood cell differential smears were established by routine techniques. Titre of IgG class serum anti-*Ch. trachomatis* antibodies was tested at serum dilutions of 1:1, 1:2, 1:4, 1:8 and 1:16 using ELISA tests on Chlamydiazyme Diagnostic Kits (Abbot Diagnostic, Germany).

Analysis of the results

The results of immunological and hematological tests (tables 1-3, figures 1-4) were evaluated in respect to the trend of alterations noted during the experiment, comparing the values obtained in immunized rabbits in consecutive days of the experiment. The results were subjected to statistical analysis using Student's t-test at $p \leq 0.05$, comparing results obtained in the immunized rabbits to those in control rabbits.

Results

Immunological tests

In rabbits immunized with *Chlamydia trachomatis*, German strain, the tendency for both increasing and decreasing values was observed in all parameters. However, significant alterations were noted in only 7 variables, including the index of ingestion (Ii), percentage of ingestion cells (%ic), values of spontaneous, stimulated and spectrophotometric NBT tests, stimulation index (SI) and coefficient of metabolic activity of PMN cells (CMAC) for the spontaneous NBT test, out of 14 evaluated variables (tables 1-3, figures 1-3). The highest frequency of the alterations, mainly in immunological indices, was noted on days 21, 28, 35 and on days 42 and 49 of the experiment and the frequency was slightly lower on days 7, 14 and 56 of the experiment. In most cases the alterations involved decreased values of the variables and in only few cases they involved augmented values of the parameter. Evaluating the alterations in time, it should be mentioned that the most persisting decrease in values pertained IP, spontaneous and stimulated NBT reduction test and values of CMAC in the spontaneous NBT test.

Analysis of immunological parameters showed that values of AC (table 1, figure 1) manifested growing tendency throughout the experiment, with a slight decrease noted on days 21 and 35 of the experiment. A slightly less pronounced increase was observed in values of Ii (table 1, figure 1), except days 14, 28 and 49, when slightly decreased values of the index were detected. In the case of Ii, a significant decrease was observed between days 7 and 28 as well as in days 49 and 56 of the experiment. In values of %ic (table 1) only a slight growing tendency was seen, with somewhat greater variations: lowered values of the parameter were noted on days 21, 35, 49 and 56 of the experiment. A significant difference in this last examined parameter, in the form of a rise, was detected on day 42 of the experiment. Having estimated values of the spontaneous NBT test (table 1, figure 2), it demonstrated an evident rising tendency with decreased values on days 28 and 49 of the experiment but a significant difference, in the form of decreased values was detected between days

Table 1. Indices of non-specific cell-mediated immunity in rabbits immunized with *Chlamydia trachomatis* – German strain

Studied parameters			Parameters in particular 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
adherence capacity (AC) (%)		$\bar{x}$	17.6	15.8	28.0	27.9	27.2	21.4	23.6	18.6	26.0	31.0	25.1	25.3	28.9	31.9	31.8	29.5	36.0	28.4
		min	16.4	12.6	24.6	26.8	26.7	18.6	19.7	14.6	23.1	29.0	21.3	21.3	26.7	28.5	24.6	26.4	31.0	25.6
		max	19.5	17.1	35.0	30.0	29.8	25.0	26.4	21.0	32.0	34.0	28.0	28.4	33.4	33.5	39.4	31.9	39.0	32.0
ingestion capacity	index of ingestion (Ii) (a.v.)	$\bar{x}$	4.1	5.7	4.7	7.0*	3.9	7.0*	4.2	9.0*	3.7	7.2*	4.2	6.2	5.4	4.7	4.9	7.0*	6.0	9.0*
		min	2.6	4.6	3.6	5.4	2.6	5.6	3.1	6.5	2.6	5.9	3.1	4.8	4.2	3.9	3.6	6.4	4.5	7.5
		max	5.1	6.4	5.4	8.2	5.7	9.2	6.1	10.3	5.8	9.1	6.1	7.2	6.7	5.4	7.2	9.5	7.9	10.5
	% ingestive cells (%ic)	$\bar{x}$	48.6	53.7	51.6	53.2	52.4	54.5	47.6	50.2	53.1	48.7	50.1	47.7	60.0*	49.2	53.5	51.7	52.5	53.7
		min	34.6	39.2	48.5	49.8	50.0	49.6	36.2	48.5	49.8	36.4	46.7	45.9	56.9	42.5	50.6	49.8	50.5	50.6
		max	59.2	65.0	56.3	56.0	55.0	68.1	58.6	52.0	65.6	51.3	65.0	49.7	75.0	66.8	55.8	55.0	55.0	55.9
NBT reduction test (a.v.)	spontaeous	$\bar{x}$	10.5	11.5	11.6	10.0	14.5	10.0	15.5	19.0*	13.5	18.3*	16.0	18.0*	16.0	21.5*	11.3	17.3*	18.4	16.6
		min	9.1	9.5	9.6	8.5	12.3	9.0	12.6	16.5	1.0	15.0	14.5	14.0	14.5	19.5	9.2	15.6	16.4	13.6
		max	12.5	13.0	13.4	13.0	19.0	14.0	17.6	24.0	15.0	20.5	19.5	21.0	20.5	24.0	15.0	21.5	21.5	20.4
	stimulated	$\bar{x}$	17.5	20.3	19.3	10.6	21.5	16.6	22.6	26.0*	21.5	25.3*	23.0	22.3	22.6	27.3*	25.0	25.0	21.0	21.3
		min	15.5	15.9	15.8	9.4	18.5	14.6	19.5	24.5	19.5	23.5	21.0	19.5	19.0	25.6	24.0	22.5	19.5	19.5
		max	21.5	25.0	22.5	12.6	25.0	21.0	26.0	28.0	24.5	28.0	25.0	23.0	25.6	29.0	26.0	27.0	26.0	23.5
	specrtrophotometric	$\bar{x}$	7.5	9.8	10.5	12.0	12.5	14.0	13.2	15.2	10.3	13.0	18.5*	15.6	21.3	19.5	19.5	17.6	15.5	17.0
		min	4.3	6.7	9.8	10.3	12.0	13.0	10.6	14.6	8.9	11.5	14.6	14.3	18.9	17.9	17.6	15.2	10.6	15.5
		max	10.3	12.0	12.3	15.2	16.0	16.0	15.2	17.2	14.0	14.6	20.3	18.5	23.4	21.0	21.0	19.8	20.3	19.0
	stimulation index (SI)	$\bar{x}$	1.6	1.7	1.6	1.1	1.5	1.6	1.4	1.4	1.6	1.4	1.4	1.2	1.4	1.3	2.2*	1.4	1.1	1.3
		min	1.2	1.5	1.4	0.9	1.2	1.4	1.0	1.2	1.4	1.0	1.1	0.9	1.2	1.2	1.8	1.0	0.9	1.0
		max	1.9	1.9	1.8	1.3	1.7	1.8	1.6	1.6	1.8	1.8	1.6	1.3	1.8	1.5	2.5	1.6	1.3	1.5
CMAC (a.v.)	spontaneous	$\bar{x}$	0.60	0.49	0.59	0.67	0.70	0.83*	0.76	1.10*	0.91	1.11*	0.89	1.16*	0.91	0.85	0.72	0.81	0.77	0.94*
		min	0.30	0.36	0.52	0.46	0.58	0.68	0.68	0.89	0.85	0.98	0.75	0.97	0.75	0.76	0.62	0.74	0.68	0.86
		max	0.89	0.58	0.71	0.74	0.91	0.97	0.86	1.25	1.00	1.23	0.98	1.24	1.10	0.97	0.85	0.92	0.94	1.10
	stimulated	$\bar{x}$	0.98	0.91	0.97	1.13	1.19	1.41	1.11	1.49	1.47	1.59	1.25	1.45	1.36	1.58	1.19	1.10	1.05	1.21
		min	0.89	0.84	0.79	0.94	1.03	1.25	1.00	1.36	1.36	1.34	1.20	1.35	1.24	1.36	1.10	1.00	0.85	0.98
		max	1.02	0.99	1.03	1.25	1.25	1.61	1.30	1.64	1.71	1.74	1.40	1.60	1.46	1.74	1.23	1.21	1.25	1.32

*Z – immunized animals; K – control animals; ↓ – day of immunization; * – statistically significant difference at $p \leq 0.05$; a.v. – absolute value.*

Table 2. Indices of humoral immunity in rabbits immunized with *Chlamydia trachomatis* – German strain

Studied parameters			Parameters in particular 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
activity of myeloperoxidase (MPO) (a.v.)	$\bar{x}$		1.6	1.8	1.6	1.9	2.0	1.8	2.1	1.9	1.7	2.0	2.1	2.2	2.0	1.9	2.2	2.0	2.1	2.0
	min		1.3	1.8	1.4	1.8	1.9	1.6	1.9	1.7	1.6	1.6	2.1	1.8	1.9	1.6	2.1	1.7	1.7	1.8
	max		2.1	1.9	1.9	2.0	2.1	2.0	2.3	2.0	1.8	2.1	2.2	2.6	2.2	2.1	2.6	2.1	2.4	2.1
lysozyme (LZM) amount (mg/l)	$\bar{x}$		6.2	6.3	6.2	6.2	5.6	6.5	6.6	6.3	7.0	6.2	6.6	7.0	6.2	6.7	6.9	6.6	6.5	6.8
	min		5.3	5.0	4.9	4.6	4.8	6.0	4.6	6.1	5.6	5.5	5.9	6.5	5.3	5.1	6.0	6.0	6.1	6,1
	max		7.0	7.2	7.5	8.1	6.8	7.0	7.1	6.7	8.0	7.6	7.6	7.5	7.0	7.4	7.5	7.5	7.0	7.4
	activity (a.v.)	$\bar{x}$	0.013	0.011	0.012	0.015	0.013	0.015	0.009	0.011	0.014	0.016	0.011	0.012	0.011	0.013	0.010	0.009	0.011	0,016
		min	0.007	0.008	0.007	0.010	0.006	0.007	0.007	0.009	0.009	0.011	0.006	0.008	0.007	0.06	0.007	0.006	0.006	0,012
		max	0.017	0.017	0.017	0.022	0.022	0.024	0.013	0.014	0.022	0.022	0.017	0.020	0.019	0.016	0.012	0.014	0.016	0,018
total Ig (in ZST units)		$\bar{x}$	30.1	31.6	29.8	32.5	27.4	32.4	26.3	28.7	30.0	31.2	29.0	30.0	32.6	31.5	29.7	31.5	34.0	32.0
		min	29.5	30.8	27.7	35.0	25.7	30.7	24.0	26.7	28.7	29.2	27.2	30.0	30.7	30.5	28.0	28.7	30.7	29.0
		max	32.0	33.5	31.0	37.2	30.0	34.5	29.2	30.0	30.7	33.5	31.5	30.7	34.5	32.5	31.2	32.5	42.0	34.2
IgG (g/l)		$\bar{x}$	13.2	15.2	14.5	15.9	14.1	14.8	15.6	15.1	14.9	15.2	16.8	16.6	15.4	15.1	15.2	14.0	14.8	14.1
		min	9.6	8.1	11.0	12.6	10.2	11.6	11.6	12.9	13.5	13.1	16.6	16.2	12.2	12.6	12.1	12.6	11.4	13.3
		max	17.6	16.9	17.6	17.9	19.1	18.5	17.4	18.3	19.1	17.3	17.1	17.6	17.6	19.4	17.2	16.8	16.4	16.2

*Z – immunized animals; K – control animals; ↓ – day of immunization; * – statistically significant difference at p ≤0.05; a.v. – absolute value.*

21 and 49 of the experiment. An analogous rising tendency, resembling that described in the latter test, was seen in values of the stimulated NBT test, with decreased values observed on days 28, 42 and 56 of the experiment and a significant decrease on days 21, 28 and 42 of the experiment. Also values of the spectrophotometric NBT test demonstrated rising tendency, with a decrease seen on days 28 and 56 and a significant increase on day 35 of the experiment. In the case of index of stimulation (SI) values a small decreasing tendency was observed throughout the experiment but an increase was noted on days 28 and 49 (in the latter day it was significant) (table 1, figure 2). CMAC values for the spontaneous NBT test manifested an increasing tendency but also slight decreases on days 7, 35 and 49 of the experiment but significant differences in the form of a decrease were detected between days 14 to 35 and on day 56 of the experiment (table 1, figure 2). A similar rising tendency was noted in CMAC values of the stimulated NBT test, in which oscillation of the values was detected: an increase on days 14, 28 and 42 and a decrease on days 7, 21, 35, 49 and 56 of the experiment. MPO activity manifested a slightly increasing tendency (table 2, figure 3) but lowered values were noted on days 28, 49 and 56 of the experiment. Throughout the experiment, amount of LZM manifested a slightly increasing tendency (table 2, figure 3) with augmented levels noted on days 21, 28 and 49 and lowered levels observed on days 14 and 42 of the experiment. On the other hand, activity of LZM demonstrated a slightly decreasing tendency with minor oscillations (except of days 21 and 28) (table 2, figure 3). Amount of IgG and total amount of serum Ig manifested a mild increase tendency (table 2, figure 3) with minor oscillations in both parameters (more pronounced in immunoglobulin levels measured in ZST units) for the entire period of the experiment.

Hematological tests

Among the 8 hematological indices in rabbits immunized with *Ch. trachomatis*, significant changes were detected in only three of them (number of neutrophilic granulocytes, of leukocytes and of basophilic granulocytes). The changes involved with equal frequency an increase and a decrease in the parameter value. The changes were noted on days 7 and 42 of the experiment and, in individual cases, on days 35, 49 and 56 of the experiment.

Table 3. Hematological indices in rabbits immunized with *Chlamydia trachomatis* – German strain

Studied parameters			Parameters in particular 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
hemoglobin (Hb) (mmol/l)		$\bar{x}$	8.0	9.0	7.5	7.0	8.5	8.5	8.0	8.0	7.5	8.0	8.0	8.5	7.5	8.0	7.0	8.0	8.0	9.5
		min	7.0	8.0	6.0	5.0	7.0	8.0	7.0	8.0	6.0	7.0	7.0	8.0	7.0	7.0	6.0	7.0	7.0	9.0
		max	9.0	10.0	9.0	9.0	10.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	8.0	9.0	8.0	9.0	9.0	10.0
erythrocytes (E) (1012/l)		$\bar{x}$	3.5	4.0	3.5	4.0	3.5	4.0	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	4.0
		min	3.0	4.0	3.0	4.0	3.0	4.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	4.0
		max	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
leukocytes (L) (109/l)		$\bar{x}$	5.0	4.5	5.0*	3.5	6.0	5.5	6.0	6.0	5.5	5.5	5.0	4.5	5.0*	3.5	6.0	6.0	7.0*	4.5
		min	3.0	4.0	3.0	3.0	3.0	5.0	4.0	5.0	4.0	5.0	3.0	4.0	2.0	3.0	4.0	5.0	3.0	3.0
		max	8.0	5.0	7.0	4.0	9.0	6.0	8.0	7.0	7.0	6.0	7.0	5.0	8.0	4.0	9.0	8.0	11.0	6.0
blood smears-1	lymphocytes		$\bar{x}$	0.78	0.76	0.77	0.79	0.79	0.79	0.76	0.79	0.79	0.82	0.68	0.76	0.73	0.76	0.74	0.67	0.73	0.73
			min	0.75	0.73	0.71	0.78	0.75	0.75	0.72	0.78	0.74	0.78	0.51	0.68	0.65	0.65	0.56	0.56	0.59	0.59
			max	0.81	0.79	0.85	0.80	0.83	0.83	0.80	0.80	0.84	0.86	0.85	0.84	0.81	0.87	0.92	0.78	0.87	0.82
	granulocytes	neutrophilic	$\bar{x}$	0.19	0.22	0.20	0.18	0.18	0.15	0.22	0.18	0.15	0.15	0.28*	0.21	0.24*	0.19	0.25	0.29*	0.23	0.21
			min	0.11	0.16	0.14	0.11	0.15	0.10	0.19	0.16	0.10	0.10	0.12	0.12	0.15	0.15	0.16	0.16	0.16	0.16
			max	0.28	0.28	0.26	0.25	0.22	0.20	0.25	0.20	0.20	0.20	0.44	0.29	0.33	0.23	0.34	0.43	0.33	0.26
		eosinophilic	$\bar{x}$	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.02	0.00	0.00	0.01	0.01	0.01
			min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01
			max	0.02	0.00	0.00	0.00	0.01	0.02	0.01	0.00	0.01	0.03	0.03	0.02	0.05	0.01	0.01	0.02	0.02	0.02
		basophilic	$\bar{x}$	0.00	0.01	0.01	0.03*	0.02	0.03	0.00	0.03	0.02	0.01	0.00	0.01	0.01	0.03*	0.00	0.01	0.01	0.01
			min	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.01	0.01
			max	0.01	0.02	0.03	0.05	0.04	0.08	0.00	0.04	0.03	0.04	0.02	0.04	0.02	0.08	0.00	0.02	0.02	0.03
	monocytes		$\bar{x}$	0.00	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.03	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00
			min	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
			max	0.01	0.01	0.02	0.00	0.00	0.03	0.04	0.00	0.08	0.01	0.03	0.00	0.00	0.03	0.01	0.01	0.01	0.01

*Z – immunized animals; K – control animals; ↓ – day of immunization; * – statistically significant difference at p ≤0,05; a.v. – absolute value.*

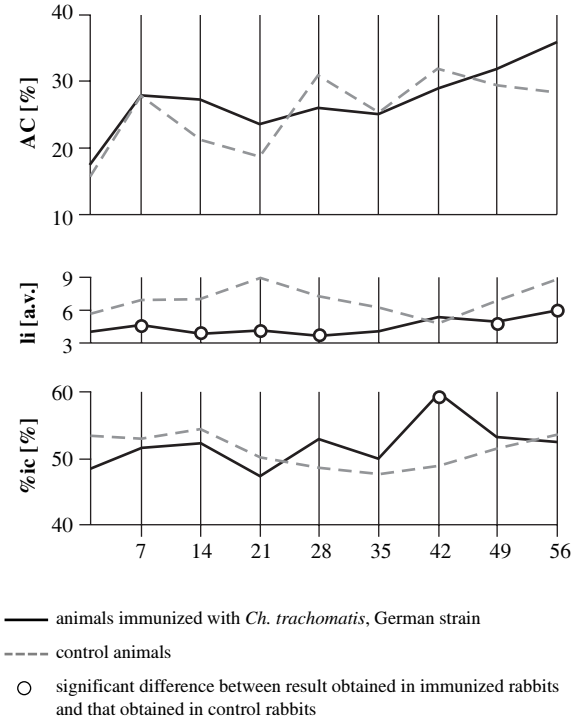

Fig. 1. Adherence capacity, index of ingestion and percent of ingestion cells in rabbits immunized with *Chlamydia trachomatis*, German strain. AC – adherence capacity, Ii - index of ingestion; %ic – percent of ingestion cells; a.v. – absolute value

Analysis of individual variables demonstrated that significant rise applied leukocytes (table 3, figure 4) on days 7, 42 and 56 and neutrophilic granulocytes on 35 and 42 days of experiment. The last parameter shows also significant decrease on 49 day, such a change is detected in basophilic granulocytes (table 3, figure 4) on 7 and 42 days after immunization.

Serological tests

In 20 of the immunized rabbits, antibodies to *Ch. trachomatis* were detected between days 21 and 35 of the experiment, at serum dilutions of 1:1, 1:2 and 1:4, and in every other rabbit they were detected also at the dilutions 1:8 and 1:16 (table 4). On day 42, in all the rabbits the antibodies were present at dilutions of 1:1 and 1:2, and in half of the rabbits at the dilution of 1:4. On days 49 and 56, half of the immunized rabbits had the antibodies even at dilutions of 1:1, 1:2 and 1:4. No such antibodies were detected in control animals.

Discussion

Analysing the results of our studies on the parameters of non-specific cell-mediated immunity we may conclude that

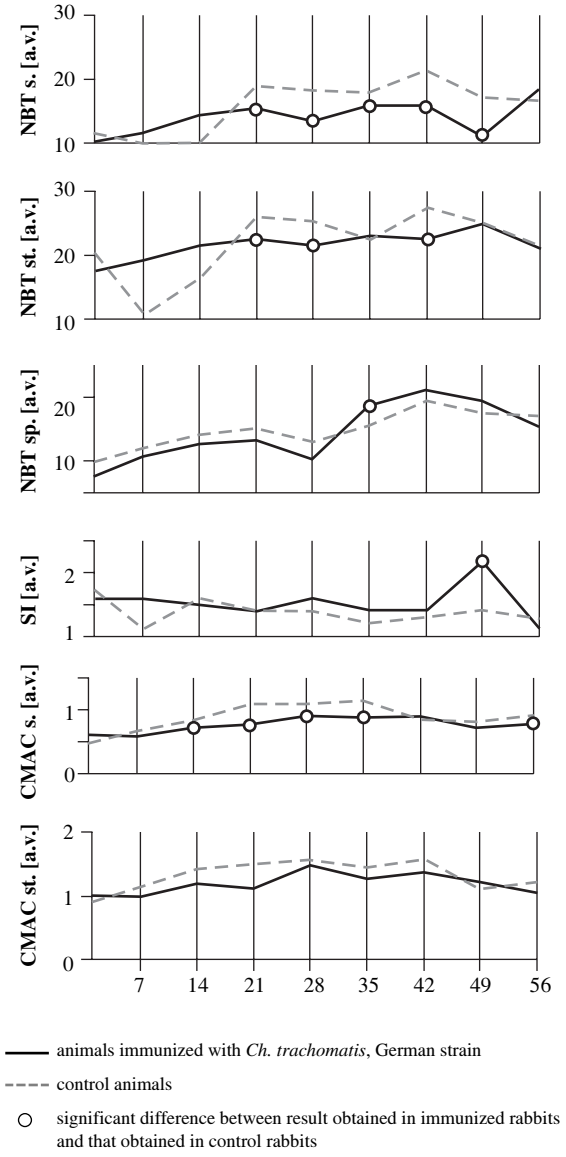

Fig. 2. Cidal ability of PMN cells measured by NBT reduction tests in rabbits immunized with *Chlamydia trachomatis*, German strain. NBT s. – spontaneous NBT reduction test; NBT st. – stimulated NBT reduction test; NBT sp. – spectrophotometric NBT reduction test; SI – stimulation index; CMAC s. – coefficient of metabolic activity of the PMN cells in spontaneous test; CMAC st. – coefficient of metabolic activity of the PMN cells in stimulated test; a.v. – absolute value

the absence of significant changes in the adherence capacity (AC) following immunization with the German strain of *Ch. trachomatis* confirms own previous results in rabbits immunized with *Ch. trachomatis* [cit. 2]. Those results were confirmed also in cattle naturally infected with *Ch. trachomatis* [3]. The latest study [3] proved, that

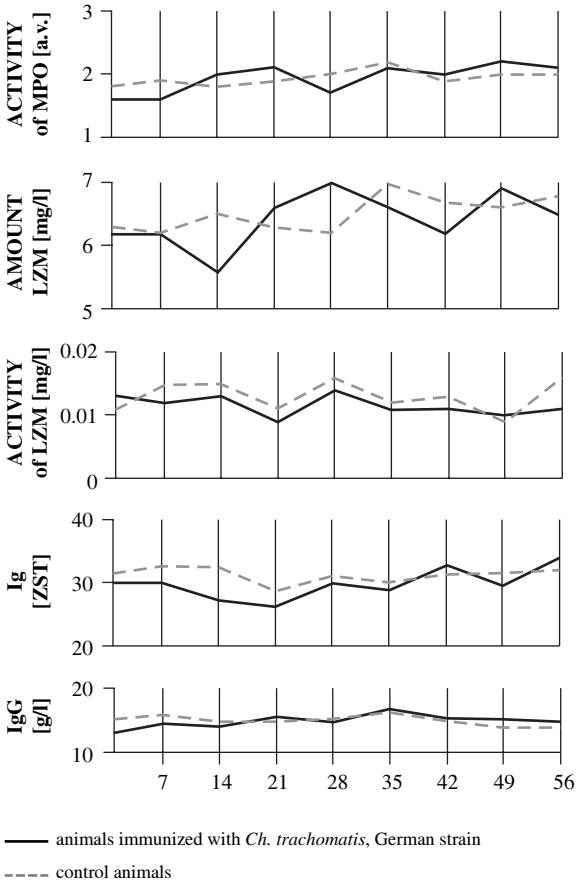

Fig. 3. Indices of humoral immunity in rabbits immunized with *Chlamydia trachomatis*, German strain. MPO – myeloperoxidase; LZM – lysozyme; Ig – immunoglobulin; IgG – immunoglobulin class G; a.v. – absolute value

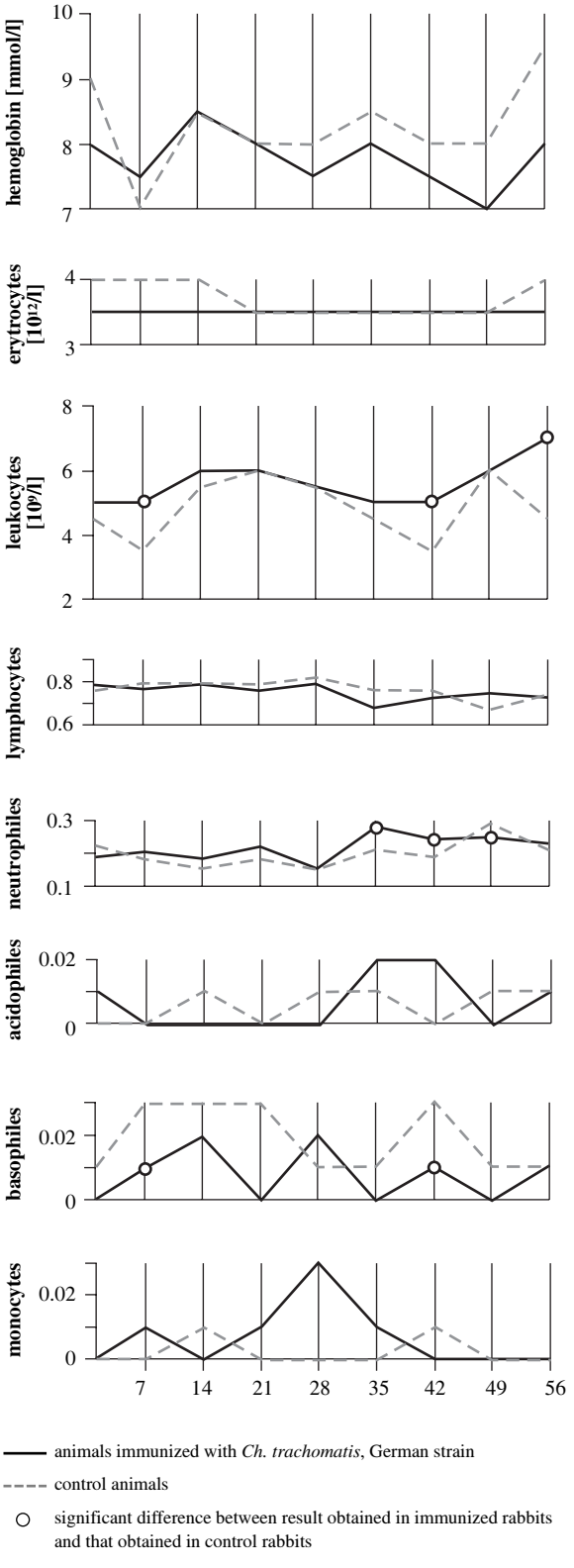

Fig. 4. Hematological indices in rabbits immunized with *Chlamydia trachomatis*, German strain

Ch. trachomatis may also infect cattle that may act as a host and not only in humans as previously thought. The absence of changes on AC values for neutrophilic granulocytes documented in the present studies and in previous observation [cit. 2] may suggest absence of changes in the capacity of PMN cells to adhere in chlamydial infections. This contradicts with observations on pigs naturally infected with other intracellular bacteria, e.g. *Salmonella choleraesuis*, where augmented AC between hours 6 and 48 following infection were noted [7].

The detected decrease in the index of ingestion (Ii) on days 7, 14, 21, 28, 49 and 56 in the studied rabbits has confirmed earlier results [cit. 2], which also demonstrated decrease in the parameter between days 14 and 28 after immunization of rabbits with *Ch. trachomatis*. A registered increase of Ii observed in humans naturally infected with *Ch. trachomatis* [8] may be the cause of other reactivity of PMN cells originating from humans and rabbits. Results obtained in humans and the documented increase in Ii

following infection with *Ch. trachomatis* [8], have been confirmed in cattle natural infected with *Ch. trachomatis* [3] and in mice in peritoneal macrophages infected with *Ch. psittaci* [9]. The lowered content of ingestion cells (%ic) in rabbits immunized with *Ch. trachomatis* on day 42 is consistent with our earlier observations [cit. 2]. The observation could not be confirmed in cattle naturally infected with *Ch. trachomatis* [3]. Summing up results of our present and earlier studies [cit. 2], and results of other authors [8, 9] on the ingestion capacity (Ii and %ic) of PMN and MN cells it can be concluded that the results confirm the rule that intracellularly inhabiting parasitic bacteria affect activity of phagocytic cells. This conclusion has also been confirmed in pigs naturally infected with *Salmonella choleraesuis* [10] and in mice experimentally infected with *Listeria monocytogenes* [11], *Salmonella typhimurium* [12] or *Francisella tularensis* [13], and thus with intracellularly inhabiting parasitic bacteria.

The detected lowered values of the spontaneous NBT test on days 21, 28, 35, 42 and 49 and of the stimulated NBT test on days 21, 28 and 42 of the experiment in studied rabbits is consistent with our results [cit. 2], although in some of the studies also increased values of the test were observed in rabbits immunized with *Ch. trachomatis* [cit. 2]. The increased values of NBT reduction test were also detected in cattle naturally infected with *Ch. trachomatis* [3]. The detected heretofore increase on day 35 of the experiment of the values of spectrophotometric NBT test and on day 49 of the stimulation index cannot be compared and are difficult to interpret due to the absence of analogous studies. However, the alterations may be thought to reflect response of PMN cells to *Chlamydia trachomatis*. The role and participation of non-specific cell-mediated immunity standing for innate immunity indices in response to immunization with *Chlamydia* in rabbits are confirmed by the results of Coefficient of metabolic activity of PMN cells in spontaneous NBT cells (CMAC) in rabbits immunized with *Ch. trachomatis*, which on days 14, 21, 28, 35 and 56 of the experiment have shown a decrease. This is consistent with results of multiple authors [cit. 2], who documented a decrease in spontaneous CMAC and absence of changes in stimulated CMAC values in rabbits immunized with *Ch. trachomatis*. It should also be added that the results of the present studies on rabbits, related to cidal activity of PMN cells as determined by NBT reduction tests, are partially in agreement with results of other authors [14], who demonstrated mostly that infection with *Ch. trachomatis* in humans resulted in augmented oxygen-dependent cidal activity of PMN cells. The data have been confirmed by studies of Ismail et al. [12], who have provided evidence for stimulation of PMN's cidal activity by such an intracellular bacteria as *Mycobacterium tuberculosis*, which secretes hyperoxide anion. The results on cidal activity of PMN cells, obtained in NBT reduction tests, prove that an infection with intracellularly residing bacteria the mechanisms are linked to oxygen-dependent cidal system,

Table 4. Serological results in ELISA test in rabbits immunized with *Chlamydia trachomatis* – German strain

***Chlamydia trachomatis* – German strain**					
Serum dilution/ Day of experiment	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**
1	–	–	–	–	–
7	–	–	–	–	–
14	–	–	–	–	–
21	+	+	+	±	–
28	+	+	+	±	±
35	+	+	+	±	±
42	+	+	±	–	–
49	±	±	±	–	–
56	±	±	±	–	–

(+) – positive reaction at all immunized rabbits; (±) – positive reaction at 50% and more immunized rabbits; (–) – negative reaction at all immunized rabbits.

as it was confirmed in mice experimentally infected with *Listeria sp*. and *Rickettsia sp*. [12].

Present results received on MPO activity, demonstrated the absence of changes, what differs from the earlier results of our team [cit. 2] and the results of Yong et al. [15], who demonstrated that *Ch. trachomatis*, biotype LGV and trachoma, induced release of myeloperoxidase from granules of human PMN cells. Also Tossi and Hammerschlag [16] proved that natural infection of humans with serotype L_2 of *Ch. trachomatis* stimulated PMN cells to demonstrate higher activity and to release MPO. Results analogous to the latter ones were also obtained in cattle naturally infected with *Ch. trachomatis*, in which augmented activities of MPO were documented [3]. On the other hand, the absence of alterations in the amount and activity of lysozyme (LZM), confirmed the results earlier obtained in rabbits [cit. 2], documenting a distinct pattern of infection-accompanying changes, as compared to bulls naturally infected with *Ch. trachomatis*, in which augmented amounts of lysozyme were detected in serum [3]. The involvement and role of MPO and LZM, presented above in anti-chlamydial defense, have been confirmed by the results on other variables forming the non-specific humoral immunity – element of anti-chlamydial innate immunity. *Chlamydia infections* in humans and animals it has been shown that levels of IFNα, TNFα [17-19] and of interleukins 1, 6 and 12 [17, 20, 21] are changing in chlamydial infections. IFNα and TNFα as well as interleukins 1, 6 and 12 were found not only to inhibit proliferation of *Chlamydia trachomatis* but also to induce their destruction in cells of humans or animals. This would indicate that eradication of the bacteria engages not only oxygen reactive form-dependent cidal systems (MPO), localised inside the phagocytes, but also

substances secreted by the cells, like cytokines, interleukins or LZM. The role of non-specific humoral immunity factors has also been confirmed in mice infected with *Mycobacterium tuberculosis*, in which bacterial destruction resulted from the joined action of not only TNFα, interleukins 1, 6 and 8 but also of reactive forms of oxygen [12].

The absence of changes in Ig and in total serum immunoglobulins G class of immunoglobulins, documented in the present studies, has confirmed observations performed in cattle naturally infected with *Ch. trachomatis* [3]. A slightly distinct pattern has been obtained by Zhang et al. [22] in mice immunized with a viable *Ch. trachomatis* antigen, in which elevated levels of IgG_{2a}, IgG_1 and IgA have been disclosed. The latter results correspond to those obtained in humans [23] and in cattle [3, 24] with natural *Ch. trachomatis* infection as well as in experimental animals (guinea pig) [25], experimentally infected with *Ch. trachomatis*, in whom/which elevated serum IgG [3, 24], IgA [3, 23] and IgM [3, 24] and secretory sIgA [25] have been documented. The data on serum Ig role in anti-chlamydial immunity in humans and animals have been confirmed by other studies on humans [35, 46, 47], in which the protection was found to be related mainly to serum IgG and IgM. Involvement of Ig in protection against *Chlamydia sp*. has also been confirmed by observations of augmented levels of serum IgG in mice experimentally infected with *Listeria sp*., *Orientia tsutsugamushi* and *Ehrlichia chaffeensis*, intracellular parasitic bacteria [12].

The evaluated relatively low and very short-lasting increase in neutrophilic granulocytes, leukocytes and decrease in basophilic granulocytes in the studied rabbits encountered difficulties reflecting very infrequent studies of the kind [cit. 2]. The studies performed on rabbits indicate that *Ch. trachomatis* induces very low changes in the number of leukocytes and of neutrophilic granulocytes. Also the specific anti-*Chlamydia* antibodies, detected in the studied rabbits from day 21 after immunization on confirm the data obtained by several authors in rabbits and in mice [cit. 2], that such antibodies are detected 14 to 21 days following immunization with *Ch. trachomatis*.

Summary

1. Immunization of rabbits with *Ch. trachomatis*, German strain, induces mainly a decrease and rarely and increase in 7 of 14 examined parameters. The changes involved ingestion capacity and potential cidal activity of PMN cells in NBT reduction test in the period between day 7-21 and day 42-56 of the experiment and induces very short-lasting alterations (increase and decrease) in number of leukocytes, neutrophilic and basophilic granulocytes (days 7, 35, 42, 49).
2. On the basis of performed studies allows one to conclude that the studied German strain of *Ch. trachomatis*, even if it represents a relatively weak immunogen among germs from *Chlamydiaceae* family [2], induces alterations mainly the decrease in immunological variables, manifested three weeks before development of positive titres of anti-*Chlamydia* antibodies.

* *Chlamydia trachomatis* described in swine now is classified as *Chlamydia suis*.

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