IMMUNOGENICITY OF COXIELLA BURNETII WHOLE CELLS AND THEIR OUTER MEMBRANE COMPONENTS

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Summary. – The immunogenicity and protective efficacy of the phase I and phase II *Coxiella burnetii* whole cells (Cb I and Cb II) and their outer membrane components (OMC), i.e. phase I trichloroacetic acid extract (TCAE), phase I 29 K protein (PRO), phase I and II lipopolysaccharides (LPS I, LPS II), polysaccharides (PS I, PS II), and lipid A (LA I, LA II), were compared. The highest immune response was observed in BALB/c mice by Cb I in both humoral immunity and lymphocyte transformation assays, and in the protective effect as well. The immune response was also significant by Cb II, but their protective capacity was low. The OMC reacted variously. Only TCAE and PRO gave a high value of humoral immunity evaluated by the serological methods. All OMC reacted in the haemolytic plaque assay giving different responses. Lymphoproliferation of splenocytes was positive with all OMC using both Cb I and Cb II antigens with the exception of PS I and PS II in the case of Cb II antigen. The induction of protection against infectious Cb I was demonstrated after immunization with TCAE, PRO, and LPS I. Other OMC did not induce protection against this agent.

Key words: *Coxiella burnetii;* outer membrane components; immunogenicity

Introduction

C. burnetii, the etiologic agent of Q fever, is an obligatory intracellular parasite of eucaryotic cells. *C. burnetii* is unique among rickettsiae in that it undergoes a virulent phase (I) to avirulent phase (II) transition upon serial laboratory passages in embryonated chicken eggs or tissue cultures (Fiset, 1957). Similarly to Gram-negative bacteria, the *C. burnetii* cell surface consists of three layers, namely, the outer membrane, the peptidoglycan layer and the inner membrane (Amano and Williams, 1984; Lambert, 1988). The structure of OMC determines the interaction between the microbe and host including the pathogenicity and immunogenicity of the agent. The OMC of *C. burnetii* have different immunogenicity. Thus, phospholipids did not induce antibodies in laboratory animals (Williams et al., 1986). LPS (I) was capable to induce antibody response and is considered as the protective immunogen (Kazár *et al.*, 1978; Marmion *et al.*, 1984). From OMC, several proteins were characterized: 29.5 K major surface protein (Williams *et al.*, 1990), 27 K protein (Müller *et al.*, 1987) and 62 K heat-shock protein (Thompson *et al.*, 1990). The 27 K protein was also cloned (Hendrix *et al.*, 1990). All these proteins were immunogenic in laboratory animals.

In present study we have investigated the immunogenic properties Cb I and Cb II and their OMC.

Materials and Methods

Animals. 10 – 12 g female BALB/c mice were used throughout. To determine the optimal dose of antigen for immunization, animals were injected intraperitoneally (ip) with 25, 50, 75, 100, 200, and 500 μg per mouse of Cb I, Cb II or their OMC. The highest response was achieved already with 100 μg antigen. Therefore, this dose was used in all immunizations.

C. burnetii strain Nine Mile, serologically in phase I and phase II (the 3rd and 163rd egg passage, respectively),was propagated in chick embryo yolk sacs. Cb I were purified by the high salt-ether technique (Ormsbee, 1962). In the case of Cb II, ether extraction was preceded by two successive treatments with 0.5% trypsin (Serva) in PBS at 37 °C for 30 mins. Then the rickettsial suspension was centrifuged and the sediment was subjected to final treatment with 0.25% trypsin at 37 °C for 30 mins. After

dilution with 0.15 mol/l NaCl, Cb II were further purified with ether.

OMC. LPS I and LPS II were isolated as described elsewhere (Toman and Kazár, 1991; Schramek and Galanos, 1981). LA I was released from LPS I under mild acidic conditions (1% acetic acid, 100 °C, 160 mins) and sedimentation by centrifugation at 10,000 × g at 5 °C for 15 mins. The sediment was resuspended in distilled water and centrifuged at 10,000 × g at 5 °C for 15 mins to remove the residual polysaccharide. This step was repeated 5 times. Finally, LA I was lyophilized. LA II was obtained from LPS II in the same way. PS I was obtained from the supernatants after centrifugation of LA I. These were pooled, concentrated by evaporation, and lyophilized. The material was purified on Sephadex G-50 column (Pharmacia) using water as the eluant. The polysaccharide fraction was collected and lyophilized, thus giving PS I. PS II was isolated from the supernatants obtained after centrifugation of LA II. The material was purified using Sephadex G-25 column (Pharmacia) similarly to PS I. The final product represented PS II. Each of the purified preparations (LPS, LA, and PS) contained about 1% proteins. TCAE was prepared from Cb I with 10% TCA at 0 °C for 45 mins (Lukáčová *et al.*, 1989). Lyophilized TCAE contained 40% sugars, 16% proteins, and about 1% phosphorus. PRO was isolated from Cb I by treating the cells with a detergent, 2% Empigen BB (Calbiochem) (Lowell *et al.*, 1988). The crude protein contained 85% proteins and 9.7% sugars. The protein was purified by chromatography on Sephadex G-100 column using water as the eluant. The eluate was collected in 6 fractions and the fraction with the highest protein content (96%) was used for immunization.

Chemical analyses. Proteins were determined spectrophotometrically with Coomassie Brilliant Blue G-250 using bovine serum albumin as a standard (Bradford, 1976). Carbohydrates were assayed by the phenol-sulphuric acid method using glucose as a reference (Dubois *et al.*, 1956). Phosphorus was assayed by the ammonium molybdate method (Lowry *et al.*, 1954).

Humoral immunity was investigated 4 weeks post immunization (p.i.) by the complement fixation (CF) (Stoker and Fiset, 1956), microagglutination (MA) (Fiset *et al.*, 1969), indirect immunofluorescence antibody tests (IFA) (Peacock *et al.*, 1983), and ELISA (Kováčová *et al.*, 1987). Hemolytic plaque assay (HPA), measuring the number of cells secreting high-efficiency IgM antibodies (Henry, 1980) was done on day 8 p.i. This method was essentially performed according to Jerne *et al.* (1963) in the modification of Gajdošová *et al.* (1977). The number of plaque forming cells (PFC) was calculated as an arithmetic mean from 10 dishes.

Lymphocyte transformation assay was done by use of mouse splenocytes. The uptake of [methyl-^{3}H] thymidine by splenocytes was used as an indicator of blastogenesis. The cell-bound radioactivity was measured in Rackbeta 1217 (LKB) liquid scintilation counter and expressed in cpm ± standard deviation (SD). The lymphoproliferative response was expressed as a stimulation index (SI), calculated as a ratio of mean cpm taken up by antigen-stimulated versus control cells. The results from triplicate experiments were calculated as described previously in detail (Gajdošová and Brezina, 1989). Statistical analysis was accomplished by the Student's t-test. Probability values of $P < 0.05$ were considered as significant.

Protection against C. burnetii infection. In order to determine the protective properties of *C. burnetii* cells and their OMC preparations, a single 100 μg dose of each antigen was given to mice, which were challenged ip 4 weeks later with 10^6 EID_{50} of phase I Nine Mile strain of *C. burnetii*. Mice inoculated with PBS only, and then infected with Cb I served as controls. Specific resistance was determined by titration of 20% spleen suspension (pooled from five mice taken on day 6 p.i.) in chicken embryo yolk sacs. The protection was considered as positive, when the yield of *C. burnetii* was at least by 2 log EID_{50} units lower than that in the control (Kazár and Schramek, 1985).

Results

Humoral immunity

In the evaluation of the effect of Cb I, Cb II and their OMC on the haemolytic antibody response, we have expressed the results as a percentage of increase of the number of plaques against the control (Fig. 1). The highest values were observed in groups immunized with Cb I, TCAE, and Cb II (increase by 99 – 110%). After administration of LPS I, LPS II, and PRO, the values increased by 63 – 67%. Lower values were for LA I and LA II (16 – 20%) and the lowest ones were for PS I and PS II (8 – 15%).

Immunization of mice with Cb I induced production of antibodies against *C. burnetii* in both phases as detected by several serological tests (Table 1). Upon immunization with Cb II, CF and MA tests showed the presence of phase II antibodies only, while the more sensitive IFA test and ELISA revealed also the presence of phase I antibodies in lower titers. TCAE and PRO were of similar immunogenicity, inducing phase I and phase II antibodies detectable by MA and IFA tests and ELISA. Among other OMC, only LPS I induced phase I and phase II antibodies detectable by IFA test and ELISA at the borderline of their sensitivity. PS I, PS II, LA I and LA II gave negative results and are not included in Table 1.

Lymphoproliferative response

The lymphoproliferative response of mice to Cb I, Cb II and their OMC is shown in Fig. 2. The highest proliferation was achieved with Cb I antigen in mice immunized with Cb I and TCAE (SI 11.96 and 11.02, respectively). A strong proliferative response was elicited also with Cb II (9.42) and PRO (8.5), followed by LPS I and LPS II (7.21 and 6.04, respectively). Responses induced by injections of LA I and LA II were even lower (3.95 and 3.84, respectively). After application of PS I and PS II, the SI values were on the borderline of positivity (2.16 and 2.25, respectively).

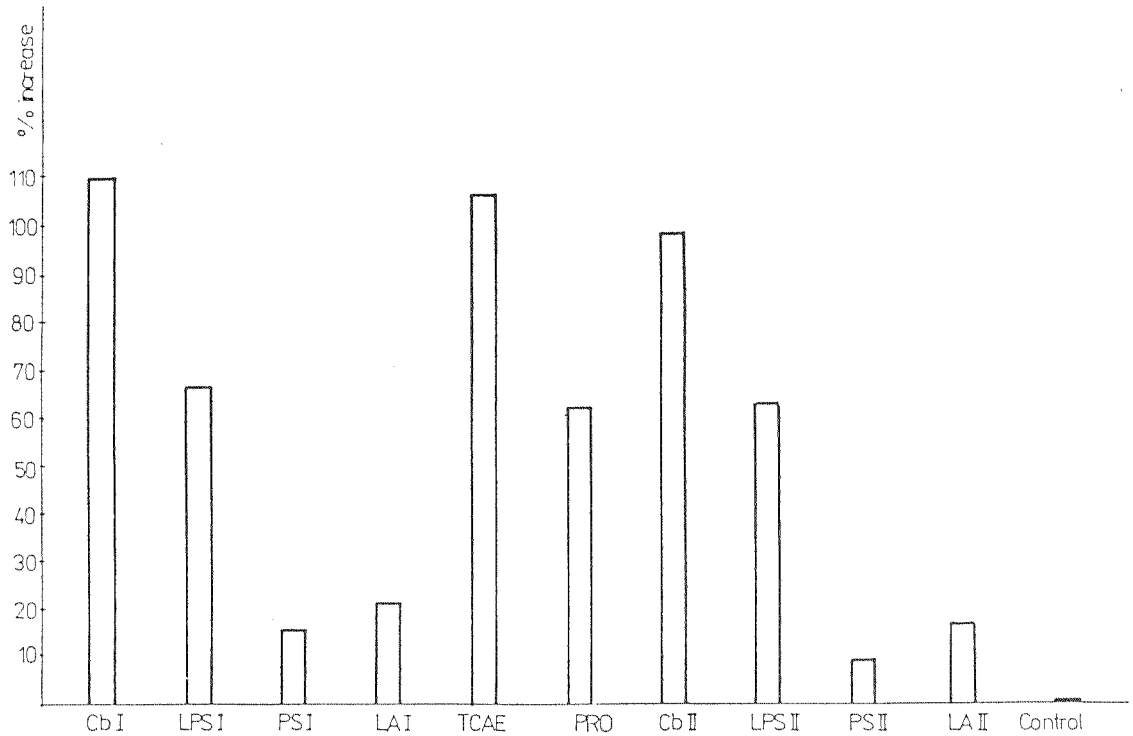

Fig. 1
Levels of IgM antibodies by Cb I, Cb II, and their OMC as detected by HPA
Abscissa: antigens used for immunization. Abbreviations: Cb I, *C. burnetii* whole cells in phase I; Cb II, *C. burnetii* whole cells in phase II; LPS I, lipopolysaccharide in phase I; LPS II, lipopolysaccharide in phase II; PS I, polysaccharide in phase I; PS II, polysaccharide in phase II; LA I, lipid A in phase I; LA II, lipid A in phase II; TCAE, trichloroacetic acid extract of Cb I; PRO, 29 K protein isolated from Cb I; OMC, outer membrane components. Ordinate: % increase against control (i.e. control corresponds to 0% increase).

Table 1. Detection of antibody response to C. burnetii antigens by CF, MA and IFA tests and ELISA after immunization of mice with Cb I, Cb II and their OMC

Test	Antigen used in the test in phase	Antibody titers after immunization with					
		Cb I	LPS I	Cb II	LPS II	TCAE	PRO
CF	I	8	<8	<8	<8	<8	<8
	II	32	<8	16	<8	<8	<8
MA	I	64	<8	<<8	<8	32	16
	II	128	<8	64	<8	32	32
IFA	I	128	16	64	<16	128	128
	II	>256	64	>256	16	64	128
ELISA	I	>3200	100	400	100	800	400
	II	>3200	100	1600	100	800	800

Antibody titers are expressed in reciprocals of dilutions. Abbreviations as in Fig. 1.

Table 2. Comparison of the protective capacity of different C. burnetii preparations in mice against challenge with 10^6 EID_{50} of phase I C. burnetii

Preparation[a]	Yields of *C. burnetii* from spleens (log EID_{50}/ml)	Difference against control (log units)[b]
Cb I	3.1	4.0
LPS I	4.7	2.4
PS I	6.7	0.4
LA I	6.6	0.5
TCAE	3.8	3.3
PRO	4.6	2.5
Cb II	5.0	2.1
LPS II	6.6	0.5
PS II	6.7	0.4
LA II	6.8	0.3
Control	7.1	–

[a] Abbreviations as in Fig. 1.
[b] Difference against control in log ≥ 2 was considered as positive.

Lymphoproliferative reponses to Cb II antigen were lower with all antigenic preparations except for LA I and LA II. In all groups the P values were from < 0.001 to < 0.05 except for PS I and PS II (P > 0.05). The values of mitogenic activities of *C. burnetii* antigens in spleen cells obtained from normal, unimmunized mice were negative (SI < 2).

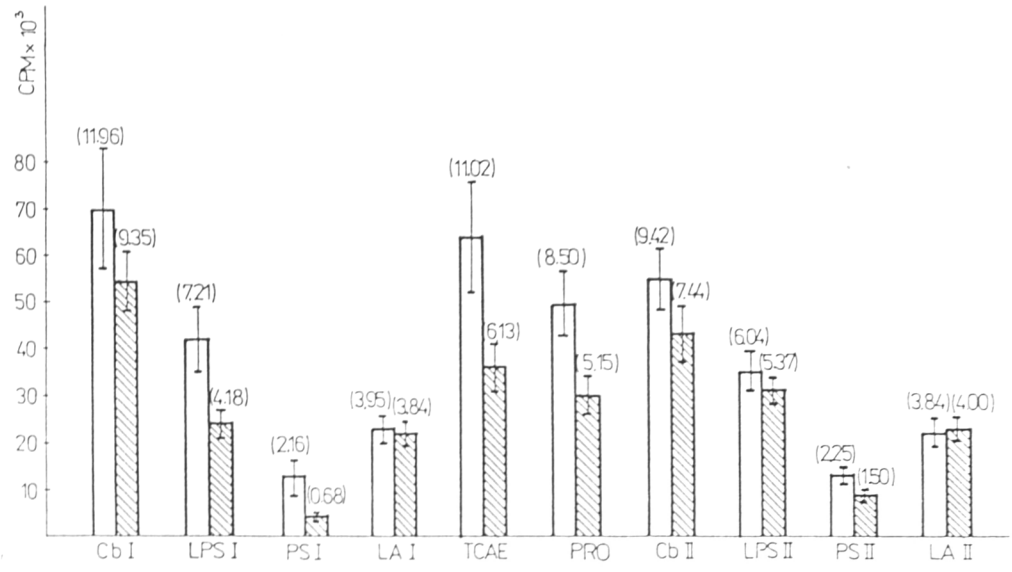

Fig. 2

Proliferative response of spleen cells obtained 4 weeks after immunization of BALB/c mice with Cb I, Cb II and their OMC

Abscissa: antigens used for immunization (abbreviations as in Fig. 1). Ordinate: mean values ± SD. SI values are in parentheses. □ Cb I; ▨ Cb II.

Protection against C. burnetii infection

The effectiveness of different *C. burnetii* antigenic preparations to induce specific resistance to phase I virulent challenge was compared (Table 2). The highest degree of resistance was observed in mice immunized with Cb I, followed by TCAE, PRO and LPS I. Cb II yielded a lower protection. Other preparations did not induce significant protection.

Discussion

In our study, the immunogenicity and protective efficacy of Cb I, Cb II and their OMC were compared in inbred female BALB/c mice immunized with one ip dose (100 μg) of a given preparation. Except for HPA, the indices of both humoral and lymphoproliferative responses were evaluated at the peak of their activities, i.e., 4 weeks p.i. (Kazár and Schramek, 1985). HPA detects the production of IgM antibodies already on day 8 after antigenic stimulus (Henry, 1980). In the assay, the highest response was obtained in mice immunized with Cb I, TCAE, and Cb II followed by LPS I, LPS II and PRO. Low values were observed after injection of LA I, LA II, PS I, and PS II.

Similar results were obtained when phase I and phase II antibody responses were measured by IFA test and ELISA. The Cb I and Cb II possessed the highest antibody-inducing capacity. Of interest was the induction of phase I antibody response by Cb II which was detectable, however, by IFA test and ELISA only. Among the OMC of Cb I, TCAE and PRO induced similar phase I and phase II antibody responses although their chemical composition was different. LPS I and LPS II were less immunogenic and induced antibody levels at the borderline sensitivity of ELISA only. Apparently, structural differences between both LPSs (see below) do not play an important role in this case. LA I, LA II, PS I, and PS II did not induce any detectable antibodies.

The highest lymphoproliferation was achieved with Cb I antigen in mice immunized with Cb I, TCAE, Cb II and PRO. High SI values were found also after immunization with LPS I and LPS II, but the SI values following application of LA I and LA II were lower. Though the latter compounds did not induce detectable antibodies, which is also in accordance with earlier reports (Galanos *et al.*, 1971; Kuhn, 1993), their lymphoproliferative responses were positive with both Cb I and Cb II antigens. Lymphoproliferative responses of PS I and PS II were positive with Cb I but negative with Cb II antigens. In general, lower values of SI were recorded with Cb II than with Cb I antigens. A 20-fold increase in blastogenic activity reported by Damrow *et al.* (1981) was not confirmed.

Each of the purified preparations (LPS, PS and LA) examined contained about 1% proteins. A possibility that the contaminating proteins in these preparations elicited the lymphoproliferative response cannot be excluded. However, their contribution appears to be minimal with respect to the results obtained. As an example may serve the low lymphoproliferative responses observed with PS I and

PS II. Williamson *et al.* (1984) tested LPS obtained from *Bacterioides fragilis* and its purified lipid A and PS components for induction of mitogenic response and polyclonal IgM synthesis. They found a more expressed mitogenic response of mouse spleen cells to PS than to LA. Similar results were obtained when polyclonal IgM synthesis was assessed. Immunogenic properties of PS and LA components from *Bordetella pertussis* LPS (Girard *et al.*, 1981) and of PS from *Haemophilus influenzae* LPS (Guenounou *et al.*, 1982; Williamson *et al.*, 1984) have been published, too. In these papers the authors reported that the mitogenic responses of mouse spleen cells were T-independent and caused by B-proliferation.

On the other hand, a growing number of examples of T-cell recognition of carbohydrate antigens has been reported (Crowle, 1988; Powderly *et al.*, 1987; Robertsson *et al.*, 1982; Shapiro *et al.*, 1982) though, to our knowledge, there has not yet been any report describing direct and unambiguous T-cell responsiveness to pure PS or LPS antigens *in vitro*. Nevertheless,there is accumulating evidence documenting the capacity of such antigens to modulate T-cell responses *in vivo* and *in vitro* (Powderly *et al.*, 1987; Shapiro *et al.*, 1982; Mehra *et al.*, 1984). In our future studies, an attempt will be made to shed more light on this problem.

In the evaluation of biological and immunological properties of *C. burnetii,* the protection against a challenge with viable rickettsiae was examined. The highest degree of resistance was observed in mice immunized with Cb I and TCAE. The protection induced by immunization with PRO, LPS I, and Cb II decreased in the given order. The other preparations were not able to induce any effective protection.

From the results reported, it is evident that the individual preparations differed considerably in their immunogenic activities. The most effective were Cb I in all parameters followed. Although both the humoral and lymphoproliferative responses of Cb II were significant, their protective capacity was low. This fact could be attributed to differences in composition and structure of LPS I and LPS II located in the outer membranes of Cb I and Cb II. It has been shown most recently, (Toman, 1992) that rough LPS II contains seven sugar residues in LA-proximal region, and the LPS I has heterogeneous O-polysaccharide chains (Toman and Kazár, 1991). It appears that the presence of O-polysaccharide chains on the surface of Cb I is important for their protective capacity. As an evidence may serve our results obtained with LPS I and LPS II. Both polymers gave similar humoral and lymphoproliferative responses but the protective capacity was found with LPS I only.

TCAE, which contains lipopolysaccharides, proteins, and phospholipids, provided both high humoral and lymphoproliferative responses, and a protective ability as well. It has been successfuly used as a vaccine against Q fever in humans (Brezina *et al.*, 1974), increasing the cellular component of immunity that can persist for many years (Gajdošová and Brezina, 1989). The immunological activity of TCAE was higher than that of PRO. Most probably, the complementary immunogenic activity of polysaccharides and proteins potentiated the final immunological activities under study. Other OMC did not give significant results with respect to immunogenicity and protective capacity.

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References

Amano, K.J., and Williams, J.C. (1984): Sensitivity of *Coxiella burnetii* peptidoglycan to lysozyme hydrolysis and correlation of sacculus rigidity with peptidoglycan-associated proteins. *J. Bacteriol.* **160**, 989–993.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal. Biochem.* **72**, 248–254.

Brezina, R., Schramek, Š., Kazár, J., and Urvölgyi, J. (1974): Q fever chemovaccine for human use. *Acta virol.* **18**, 269.

Crowle, A.J. (1988): Immunization against tuberculosis: what kind of vaccine ? *Infect. Immun.* **56**, 2769–2773.

Damrow, T.A., Cantrell, J.L., and Williams, J.C. (1981): Modification of immune competence in mice by Q fever vaccine. In W. Burgdorfer and R.L. Anacker (Eds): *Rickettsiae and Rickettsial Diseases.* Academic Press, Inc., New York, pp. 115–125.

Dubois, M., Gilles, K.R., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956): Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**, 350–356.

Fiset, P. (1957): Phase variation of Rickettsia (*Coxiella*) burnetii. Study of antibody response in guinea pig and rabbits. *Canad. J. Microbiol.* **3**, 435–445.

Fiset, P., Ormsbee,R.A., Silberman, R., Peacock, M., and Spielman, S.H. (1969): A microagglutination technique for detection and measurement of rickettsial antibodies. *Acta virol.* **13**, 60–66.

Gajdošová, E., Mayer, V., and Pogády, J. (1977): Depression of the primary humoral antibody response by trifluoroperazine, a phenothiazine derivative. *Biologia,* **32**, 679–687.

Gajdošová, E., and Brezina, R. (1989): Cell-mediated immune response to *Coxiella burnetii* antigens in Q fever convalescents and vaccinees. *Acta virol.* **33**, 474–481.

Galanos, C., Lüderitz, O., and Westphal, O. (1971): Preparation and properties of antisera against the lipid-A component of bacterial lipopolysaccharides. *Eur. J. Biochem.* **24**, 116–122.

Girard, R., Chaby, R., and Bordenave, G. (1981): Mitogenic response of C3H/HeJ mouse lymphocytes to polyanionic polysaccharides obtained from *Bordetella pertussis* endotoxin and from other bacterial species. *Infect. Immun.* **31**, 122–128.

Guenounou, M., Raichvarg, D., Hatat, D., Brossard, C., and Agneray, J. (1982): *In vitro* immunological activities of the polysaccharide fraction from *Haemophilus influenzae* type a endotoxin. *Infect. Immun.* **36**, 603–608.

Hendrix, L.R., Samuel, J.E., and Mallavia, L.P. (1990): Identification and cloning of a 27 kDa *Coxiella burnetii* immunoreactive protein. *Annals of the New York Academy of Sciences* **590**, 534–540.

Henry, C. (1980): Hemolytic plaque assays. In B.B.Mishell and S.M.Shiigi (Eds): *Selected Methods in Cellular Immunology*. Freeman W.H. and Co., San Francisco, pp. 69-123.

Jerne, N. K., Nordin, A.A., and Henry, C. (1963): The agar plaque technique for recognizing antibody-producing cells. In B.Amos and H.Koprowski (Eds): *Cell-bound antibodies*. Wistar Institute Press, Philadelphia, pp. 109–122.

Kazár, J., Schramek, Š., and Brezina, R. (1978): Immunological properties of the lipopolysaccharide-protein complex of *Coxiella burnetii*. *Acta virol.* **22**, 309–315.

Kazár, J., and Schramek, Š. (1985): Relationship between delayed hypersensitivity reaction and resistance to virulent challenge in mice immunized with different *Coxiella burnetii* antigenic preparations. In J.Kazár (Ed): *Rickettsiae and Rickettsial Diseases*. Proceedings of IIIrd International Symposium, Slovak Academy of Sciences, Bratislava, pp. 282–288.

Kováčová, E., Gallo, J., Schramek, Š., Kazár, J., and Brezina, R. (1987): *Coxiella burnetii* antigens for detection of Q fever by ELISA in human sera. *Acta virol.* **31**, 254–259.

Kuhn, H-M. (1993): Immune response of rabbits to lipid A: Influence of immunogen preparation and distribution of various lipid A specificities. *Infect. Immun.* **61**, 680–688.

Lambert, P. A. (1988): Enterobacteriaceae: Composition, structure and function of the cell envelope. *J. Applied Bacteriol.* Symposium supplement, **65**, 21 S–34 S.

Lowell, G.H., Smith, L.F., Seid, R.C., and Zollinger, W.D. (1988): Peptides bound to proteosomes via hydrophobic feet become highly immunogenic without adjuvants. *J. exp. Med.* **167**, 658–663.

Lowry, O.H., Roberts, N.R., Leiner, K.Y., Wu, M.L., and Farr, A.I. (1954): The quantitative histochemistry of brain. *J. biol. Chem.* **207**, 1–17.

Lukáčová, M., Brezina, R., Schramek, Š., and Pastorek, J. (1989): Chemical composition of phase I *Coxiella burnetii* soluble antigen prepared by trichloroacetic acid extraction. *Acta virol.* **33**, 75–80.

Marmion, B.P., Ormsbee, R.A., Kyrkou, M., Wright, J., Worswick, S., Cameron, S., Esterman, A., Ferry, B., and Collins, W. (1984): Vaccine prophylaxis of abattoir-associated Q fever. *Lancet* **II**, 1411–1414.

Mehra, V., Brennan, P.J., Rada, E., Convit, J., and Bloom, B.R. (1984): Lymphocyte suppression in leprosy induced by unique *M.leprae* glycolipid. *Nature* (London) **308**, 194–196.

Müller, H.P., Schmeer, N., Räntamaki, L., Semler, B. and Krauss, H. (1987): Isolation of a protein antigen from *Coxiella burnetii*. *Zbl. Bakt. Hyg.* A **265**, 277–289.

Ormsbee, R.A. (1962): A method of purifying *Coxiella burnetii* and other pathogenic rickettsiae. *J. Immunol.* **88**, 100–108.

Peacock, M.G., Philip, R.N., Williams, J.C. and Faulkner, R.S. (1983): Serological evaluation of Q fever in humans: enhanced phase I titres of immunoglobulins G and A diagnostic for Q fever endocarditis. *Infect. Immun.* **41**, 1089–1098.

Powderly, W.G., Pier, G.B., and Markham, R.B. (1987): *In vitro* T cell-mediated killing of *Pseudomonas aeruginosa*. V. Generation of bactericidal T cells in nonresponder mice. *J. Immunol.* **138**, 2272–2277.

Robertsson, J.A., Svenson, S.B., and Lindberg, A.A. (1982): *Salmonella typhimurium* infection in calves: delayed specific skin reactions directed against the O-antigenic polysaccharide chain. *Infect. Immun.* **37**, 737–748.

Schramek, Š., and Galanos, C. (1981): Lipid A component of lipopolysaccharides from *Coxiella burnetii*. *Acta virol.* **25**, 230–234.

Shapiro, M.E., Onderdonk, A.B., Kasper, D.L., and Finberg, R.W., (1982): Cellular immunity to *Bacterioides fragilis* capsular polysaccharides. *J. exp. Med.* **155**, 1188–1197.

Stoker, M.G.P., and Fiset, P. (1956): Phase variation of the Nine Mile and other strains of *Rickettsia burnetii*. *Can. J. Microbiol.* **2**, 310–321.

Thompson, H.A., Bolt, C.R., Hoover, T., and Williams, J.C. (1990): Induction of heat-shock proteins in *Coxiella burnetii*. *Annals of the New York Academy of Sciences*, **590**, 127–135.

Toman, R., and Kazár, J. (1991): Evidence for the structural heterogeneity of the polysaccharide component of *Coxiella burnetii* strain Nine Mile lipopolysaccharide. *Acta virol.* **35**, 531–537.

Toman, R. (1992): Structural characterization of lipid A proximal carbohydrate region of a lipopolysaccharide from *Coxiella burnetii* strain Nine Mile in phase II. *Glycobiology* **2**, 463.

Williams, J.C., Damrow, T.A., Waag, D.M., and Amano, K.I. (1986): Characterization of a phase I *Coxiella burnetii* chloroform-methanol residue vaccine that induce active immunity against Q fever in C57BL/10ScN mice. *Infect. Immun.* **51**, 851–858.

Williams, J.C., Hoover, T.A., Waag, D.M., Banerjee-Bhatnagar, N., Bolt, C.R., and Scott, G.H. (1990): Antigenic structure of *Coxiella burnetii*. A comparison of lipopolysaccharide and protein antigens as vaccines against Q fever. *Annals of the New York Academy of Sciences*, **590**, 370–380.

Williamson, S. I., Wannemuehler, M. J., Jirillo, E., Pritchard, D.G., Michalek, S. M., and McGhee, J. R. (1984): LPS regulation of the immune response: Separate mechanisms for murine B cell activation by lipid A (direct) and polysaccharide (macrophage-dependent) derived from bacterioides LPS. *J. Immunol.* **133**, 2294–2300.