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IMMUNITY IN Q FEVER

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Summary. — Post-infection and post-vaccination immune mechanisms in Q fever are summarized. Whereas cell-mediated immunity has been found to play a crucial role in developing resistance to *Coxiella burnetii* infection, data on the role of specific antibodies in Q fever immunity are controversial. The functional state of immunocompetent cells and professional phagocytes seems to be decisive for the persistence of *C. burnetii* within phagocytic cells and for the control of Q fever at the host level. Defects of cellular immunity, immune complex formation, and immune response modulation by *C. burnetii* isolates differing in plasmid composition or LPS antigenic structure are implicated as aetiological factors. Immunogenicity and reactogenicity of three possible vaccine candidates (phase I chloroform-methanol treated and untreated corpuscular vaccine, and phase I soluble chemovaccine) for Q fever prophylaxis is discussed, stressing the need for developing suitable models and defined experimental conditions enabling to compare and evaluate the results obtained in different laboratories.

Key words: Q fever; immunity; C. burnetii persistence; vaccination

The renaissance of rickettsiology during the last decade was accompanied by an accumulation of new data on immunity in rickettsial diseases in general and in Q-fever in particular. Due to the use of more defined experimental conditions and novel techniques, our current knowledge on antigenic structure of *Coxiella burnetii*, immune mechanisms in Q-fever and Q-fever immunoprophylaxis has surpassed the expectations of the mid-seventies, when only limited information on immunity in Q-fever was available (presented at the 2nd International Symposium on Rickettsiae and Rickettsial Diseases, J. Kazár, Ed., 1978).

The outcome of Q-fever, i.e. whether the *C. burnetii* exposed subject develops inapparent or overt acute and eventually chronic infection, is obviously determined by both the agent virulence potential and the host defence

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mechanisms. Not only different virulence of *C. burnetii* strains can be attributed to their phase state (Kazár *et al.*, 1974; Brezina, 1978), but detection of chromosomal and plasmid DNA restriction fragment length polymorphism (O'Rourke *et al.*, 1984; Samuel *et al.*, 1985; Vodkin and Williams, 1986; Vodkin *et al.*, 1986) demonstrates differences among phase I or phase II *C. burnetii* strains from various geographical locations and environmental sources, even though these various isolates appeared to be serologically similar. Intrastrain heterogeneity in structure and antigenicity of phase I lipopolysaccharide (LPS) of *C. burnetii* (Hackstadt *et al.*, 1985; Hackstadt, 1986) indicates that different *C. burnetii* isolates not only may have unique virulence characteristics, but also may vary antigenically.

Cell mediated immunity

As host defence mechanisms in Q-fever are concerned, the accumulated data stress the role of cell-mediated immunity (Baca and Paretsky, 1983). Briefly, cellular immune responses such as lymphocyte blast transformation and macrophage migration inhibition were demonstrated in the absence of detectable antibody response (Jerrells *et al.*, 1975; Kishimoto and Burger, 1977; Kishimoto *et al.*, 1978*a*), immunity was transferred passively not only by serum but also by lymphocytes from immune mice (Kazár *et al.*, 1977a), absence of the clearance of *C. burnetii* by athymic nude mice was observed in the presence of antibodies (Kishimoto *et al.*, 1978*b*), *C. burnetii* infection was enhanced in cyclophosphamide-treated laboratory animals in which cellular immunity was suppressed (Ascher *et al.*, 1980; Kazár *et al.*, 1982*a*), and recently both sensitive and resistant mice infected with *C. burnetii* developed similar antibody levels, though the course of infection was quite different (Scott *et al.*, 1987). Resistance to virulent challenge in mice immunized with different *C. burnetii* antigenic preparations correlated better with a delayed type hypersensitivity reaction than with antibody response (Kazár and Schramek, 1985; Kazár *et al.*, 1986) and skin test rather than antibody response was demonstrated as a useful indicator of a previous Q-fever exposure (Kudelina and Kambaratov, 1969; Kambaratov *et al.*, 1971; Terentiev and Zeleniuk, 1973; Cracea *et al.*, 1976, 1978; Kazár *et al.*, 1982*b*, 1984; Marmion *et al.*, 1984).

Effect of antibodies

Though the protective effect of phase I antibody in experimental animals was demonstrated as long as 30 years ago (Abinanti and Marmion, 1957), the development of resistance as detected by an increased clearance rate of *C. burnetii* from the spleens of infected mice could be achieved when immune serum was transferred simultaneously with *C. burnetii* or 24 hours before, but not 24 hours after *C. burnetii* challenge, and immune serum had no effect on rickettsial multiplication in athymic mice (Humphres and Hinrichs, 1981). We found that serum containing phase I antibody protected mice against virulent challenge, but was not able to neutralize phase I *C. burnetii* infectivity when tested in yolk sac of embryonated eggs and in cell cultures. The

titre of protective antibodies corresponded to the titre of phase I opsonins rather than to phase I agglutinating and complement-fixing antibodies (Kazár *et al.*, 1973). From this data it follows that only professional phagocytes can be influenced by specific Q-fever antibody which may then accelerate the initial reactions of the inductive phase of the cellular immune response (Humphres and Hinrichs, 1981). In this connection it is worth to mention the demonstration of antibody-dependent cellular cytotoxicity of *Coxiella burnetii*-infected macrophage target cells which may participate in the primary defense, or alternatively, may facilitate the dissemination of *C. burnetii* (Koster *et al.*, 1984).

There is no doubt of the opsonizing effect of a Q-fever immune serum since the treatment of phase I *C. burnetii* cells (and to a lesser extent also of phase II cells) with sera containing corresponding phase specific antibodies enhanced phagocytosis of the rickettsiae by both polymorphonuclear leukocytes (Brezina and Kazár, 1963, 1965; Wisseman *et al.*, 1967) and macrophages (Kazár *et al.*, 1975; Kishimoto and Walker, 1976; Kishimoto *et al.*, 1976). At the same time, the results on the fate of opsonized *C. burnetii* within macrophages were controversial. On one hand, potentiation of destruction of either rickettsial phase by immune serum in normal macrophages and of phase I *C. burnetii* by macrophages from phase I immunized animals in the absence of immune serum were reported (Kishimoto *et al.*, 1976, 1977; Little *et al.*, 1983). On the other hand, no such effect of immune serum was observed by other authors (Hinrichs and Jerrells, 1976; Skultetyová *et al.*, 1978). It was suggested that the presence of antibody to *C. burnetii* can be even detrimental to the sensitive host by increasing uptake and placement of the agent in the phagolysosomes, thereby facilitating the spread of infection (Baca *et al.*, 1984). However, the growth of phagocytized *C. burnetii* was suppressed in normal guinea pig macrophages treated with a lymphokine-containing supernatant or cultured with immune lymphocytes (Hinrichs and Jerrells, 1976) probably due to interferon gamma, which was found to inhibit *C. burnetii* growth in mouse fibroblasts and could be a part of crude lymphokine preparation (Turco *et al.*, 1984). In this connection, the capability of *C. burnetii* or its fractions to induce interferon, stimulate natural killer cells, activate macrophages and modify non-specific host resistance (Kazár, 1966; Brezina *et al.*, 1968; Kelly *et al.*, 1976; Kelly, 1977; Paquet *et al.*, 1978; Clark, 1979; Kazár and Schramek, 1979; Kishimoto and Gonder, 1979; Damrow *et al.*, 1981; Macela *et al.*, 1985) should also be considered.

It is possible that specific antibody exerts its protective effect only in the presence of activated macrophages (Humphres and Hinrichs, 1981), which can be crucial in eliminating the agent adapted to thrive in phagolysosomal vacuoles of host cells (Hackstadt and Williams, 1981). On the other hand, the presence of specific phase I antibody may promote activation of the phagocyte metabolism, namely superoxide anion production and monophosphate shunt stimulation (Ferenčík *et al.*, 1984). The functional state of immunocompetent cells and professional phagocytes can be of paramount importance in the control of Q-fever at the host level, e.g. activated T-lymphocytes

enhance the microbicidal activity of macrophages against *C. burnetii* (Kishimoto *et al.*, 1978*a*).

Persistence of C. burnetii

Persistence of *C. burnetii* within phagocytic cells (Khavkin and Amosenkova, 1969; Ariel *et al.*, 1973; Baca *et al.*, 1981; Hackstadt and Williams, 1981) and possibly in other cell types apparently accounts for development of chronic Q-fever in man manifesting by granulomatous hepatitis and/or endocarditis (Turck *et al.*, 1976) and for shedding the agent by placental tissues, birth fluids and excreta of infected livestock during parturition or abortion. Intraphagolysosomal survival of *C. burnetii* can be explained by its superoxide dismutase and catalase activities (Akporiaye and Baca, 1983). Peptidoglycan-protein complex, resistant to hydrolysis by proteolytic enzymes may play an important role in the rigidity of the cell wall in withstanding microbicidal conditions of the phagolysosome (Amano and Williams, 1984*a*). Endospores formation in a "life cycle" of *C. burnetii* may also be taken into account (McCaul and Williams, 1981). The "steady state" established between this intracellular parasite and its host can be disbalance, leading to the reactivation of *C. burnetii* infection, as demonstrated in laboratory animals by immunosuppressive effects of X-irradiation, corticosteroids cyclophosphamide and by hormonal effects during pregnancy (Sidwell *et al.*, 1964*a*, 1964*b*; Sidwell and Gebhardt, 1966; Tokarevich, 1979; Kazár and Kováčová, 1983).

Defects of cellular immunity found in Q-fever endocarditis patients, such as specific lymphocyte unresponsivness to *C. burnetii* antigens due to the proliferation of suppressor T-cells resulting in rickettsemia and continuous exposure to *C. burnetii* antigens can contribute to development of this form of chronic Q-fever infection (Koster *et al.*, 1985*a*, 1985*b*). Constant antigenic stimulation leads apparently to increased levels of gamma globulin, high phase I antibody titre reflecting chronic disease (Turck *et al.*, 1976) that may develop into endocarditis (Kazár *et al.*, 1977*b*; Kimbrough *et al.*, 1979). The enhanced IgG antibody levels with the presence of IgA antibodies to both phase I and phase II *C. burnetii* antigens as detected by the indirect microimmunofluorescence test are considered diagnostic for Q-fever endocarditis (Peacock *et al.*, 1983). However, high phase I antibody levels in chronic Q-fever endocarditis may reflect immunopathological changes, such as immune complex formation, rather than manifestation of immunity (Kazár *et al.*, 1977*b*). In fact, immune complexes have been detected in both laboratory infected guinea pigs (Williams *et al.*, 1981*a*) and naturally infected humans (Lumio *et al.*, 1981). Last but not least, the possibility of immune response modulation by *C. burnetii* endocarditis isolates which differ from the strains of other origin in plasmid homology (Samuel *et al.*, 1985) or LPS antigenic structure (Hackstadt, 1986) should be also taken into consideration.

Immunoprophylaxis of Q-fever

Development of knowledge on *C. burnetii*, namely after discovery of phase variation phenomenon (Stocker and Fiset, 1956), was followed by trials to introduce efficient vaccines for Q-fever immunoprophylaxis. Corpuscular vaccines used during the first 30 years following the description of Q-fever and its rickettsial aetiology were either of low immunogenicity (probably phase II cells) or of high reactogenicity (phase I cells), the untoward postvaccination reactions having occurred especially in individuals sensitized previously with *C. burnetii* (Marmion, 1967). The use of attenuated M-44 *C. burnetii* strain, originally proposed for Q-fever immunoprophylaxis in the Soviet Union (Genig *et al.*, 1965), was later abandoned because of its dubious phase state, possibility of reactivation of infection and ability to produce pathological changes in experimental animals (Johnson *et al.*, 1976, 1977).

As follows from the recent Lancet's Editorial (1984), an effective Q-fever vaccine must contain or consist of LPS-protein complex typical of phase I *C. burnetii* organisms (Schramek, 1978), since phase I cells are of much higher protective potency against phase I virulent challenge than phase II cells (Ormsbee *et al.*, 1964; Votruba *et al.*, 1985). Formalin-killed phase I *C. burnetii* cells grown in yolk sac of embryonated eggs are highly immunogenic (Kazár *et al.*, 1974; Spicer and DeSanctis, 1976), but they can induce pathological reactions (Baca and Paretsky, 1983) and possess immunomodulatory properties (Kazár and Schramek, 1984*a*) which may account for adverse effects occurring in postvaccination trials. However, higher doses of phase I cells were required to induce pathological reactions than immunity (Kazár and Schrámek, 1984*a*). Chloroform-methanol (CM) treatment of phase I cells abolished their ability to cause hepatosplenomegaly and death in mice (Williams and Cantrell, 1982; Kazár *et al.*, 1983*a*) and their negative modulatory effects on lymphocyte responsivness to mitogens (Damrow *et al.*, 1985). Such a treatment also resulted in the absence of gross pathology, namely liver necrosis (Williams and Cantrell, 1982) as well as histological and ultrastructural changes (Jakubovsky *et al.*, 1985; Kokorin *et al.*, 1985). Dermal granulomas in guinea pigs caused by phase I cells (Ascher *et al.*, 1983*b*) did not appear after intradermal administration of CM-treated phase I cells, the CM extraction preserving at the same time determinants responsible for elicitation of specific delayed type hypersensitivity reaction and for lymphocyte stimulation equivalent to that of whole phase I *C. burnetii* cells (Ascher *et al.*, 1983*c*). Sensitization of mice to bacterial endotoxin (Schrámek *et al.*, 1984), rickettsial toxin (Kazár *et al.*, 1984*b*) and stimulation of nonspecific host resistance (Kazár and Schramek, 1984*a*; Macela *et al.*, 1985) was also reduced by the CM treatment.

The CM extract itself did not exert adverse reactions in endotoxin-nonresponder mice, but reconstitution of CM residue with the CM extract restored the immunopathological reactions that were associated with the whole phase I *C. burnetii* cells (Williams *et al.*, 1986*a*). Since the CM residue from phase I cells did not induce lymphocyte unresposivness and was highly protective against a lethal intraperitoneal phase I *C. burnetii* challenge, it was suggested

as a potential candidate to replace phase I *C. burnetii* cells in the use for human vaccination (Williams *et al.*, 1986*a*).

Another approach to avoid adverse effects of phase I corpuscular vaccine was by extraction of a phase I antigenic component from phase I *C. burnetii* cells by trichloroacetic acid (Brezina and Úrvölgyi, 1961). The soluble extract characterized later as LPS-protein complex (Schramek, 1978) was recommended for Q-fever vaccination by subcutaneous route (Cracea *et al.*, 1973; Brezina *et al.*, 1974) and successfully used in several hundreds persons professionally exposed to Q-fever in Czechoslovakia (Kazár *et al.*, 1982*b*). This soluble chemovaccine was of a low reactogenicity and the occurrence of postvaccination reactions could be reduced by exclusion from the vaccination of seropositive and skin test positive subjects as similar as in the field vaccination trial using a low dose (30 μg) of phase I corpuscular vaccine (Marmion *et al.*, 1984).

Efficacy of vaccination in humans

Skin test was found superior to serological tests in assessment of both prevaccination exposure and postvaccination immunity (Ascher *et al.*, 1983*a*; Kazár *et al.*, 1982*b*, 1984; Marmion *et al.*, 1984). In subjects vaccinated with phase I corpuscular vaccine, the skin test correlated well with lymphocyte blast transformation (Ascher *et al.*, 1983*a*). It did so also in those vaccinated with the soluble Q-fever vaccine, whereas no correlation between the skin test positivity and inhibition of migration of peripheral blood leukocytes was noticed (Kazár *et al.*, 1984). In Australian study positive lymphocyte proliferative responses were maintained for at least 96 weeks after vaccination with phase I whole cell vaccine and were found more frequently positive than skin test positivity (Izzo *et al.*, 1988). Out of serological tests, immunofluorescence antibody assay was more sensitive than complement-fixation and microagglutination (Ascher *et al.*, 1983*a*; Kazár *et al.*, 1983*b*; Worswick and Marmion, 1985).

Vaccine efficacy can be determined either on humans exposed to natural *C. burnetii* infection or in experimentally infected animals. Except for American study in which immunity to respiratory challenge with a large dose of *C. burnetii* was proved on volunteers given one dose of 30 μg of phase I corpuscular vaccine (Fiset, personal communication), the efficiency of corpuscular and soluble vaccine has been estimated based on the reduced frequency of Q-fever among vaccinees exposed to the infection in the laboratory or in the abattoir (Brezina *et al.*, 1974; Kazár *et al.*, 1982*b*; Marmion *et al.*, 1984). All Q-fever vaccine candidates, i.e. CM-treated or untreated phase I cells and soluble chemovaccine, were found effective against virulent intraperitoneal challenge in mice (Kazár and Schramek, 1985) and aerosol challenge in guinea pigs (Votruba *et al.*, 1985). However, resistance to virulent challenge appeared earlier and lasted longer in both mice and guinea pigs immunized with the whole cell phase I vaccine than in those given CM-treated phase I cells or soluble vaccine (Kazár *et al.*, 1986), and higher doses of CM-treated than untreated *C. burnetii* preparations were required to induce anti-

body response. delayed type hypersensitivity reaction and protection from *C. burnetii* infection (Kazár *et al.*, 1987). Thus, although there is no doubt about the reduced reactivity and side effects of phase I *C. burnetii* CM residue which has been also found highly immunogenic and protective in other studies (Ascher *et al.*, 1983*c*; Williams *et al.*, 1986*a*), its use for a large scale vaccination of man should be carefully considered.

Because in evaluating the suitability of Q-fever vaccine and immunity in *C. burnetii* infection not only type and dose of the vaccine tested, but also the species of experimental animals, the routes of their immunization and infection may play a role (Kazár *et al.*, 1986), some discrepancies in the results obtained in different laboratories could be attributed to different experimental conditions employed. Hence the use of antigenic preparations devoid of host cell components and characterized by monoclonal antibodies, of laboratory animal strains with defined sensitivity to infection and of reliable techniques for immunity evaluation as pointed out by the Williams group (Williams and Cantrell, 1982; Williams *et al.*, 1981*b*, 1984, 1986*a*, 1986*b*, 1986*c*; Scott *et al.*, 1987) is necessary to achieve comparable results. To assess postvaccination immunity, other serological tests such as opsonization-phagocytosis reaction (Kazár *et al.*, 1973), mouse protection test and radioimmunoassay or formation of gamma interferon by peripheral white blood cells of vaccinees on stimulation with *C. burnetii* antigens (Marmion, personal communication), skin test with detection of subsequent antibody recall (Cracea *et al.*, 1977; Peacock *et al.*, 1978; Kazár *et al.*, 1984) and enzyme-linked immunosorbent assay (Williams *et al.*, 1986*b*) can be recommended. Further studies are necessary to find out how long the postvaccination protection lasts and whether booster doses of the vaccine are required. It is possible that protection might be boosted by periodic natural exposure to *C. burnetii* without clinical illness (Lancet, Editorial, 1984), that longer duration of immunity observed in laboratory animals given whole cell phase I vaccine might be caused by persisting antigenic stimulus, though this vaccine was not able to affect already established *C. burnetii* infection (Kazár and Kováčová, 1983).

Recent data on genetic heterogeneity (Samual *et al.*, 1985; Vodkin *et al.*, 1986) and antigenic variation in the phase I LPS (Hackstadt *et al.*, 1985; Hackstadt, 1986) among *C. burnetii* isolates, especially those from chronic Q-fever human cases, stress a need for thorough cross-protection studies with *C. burnetii* strains in question and for decision whether monovalent or polyvalent Q-fever vaccine prepared from *C. burnetii* strains from acute or chronic Q-fever cases should be used (Kazár and Řeháček, 1987). Results of our preliminary experiments revealed cross-protection and similar protective potency between phase I *C. burnetii* strains Nine Mile and Priscilla, the latter being isolated from chronic Q-fever endocarditis (Brezina *et al.*, to be published). Though, it is believed that all *C. burnetii* strains isolated in nature are in phase I (Lancet, Editorial, 1974), recent findings indicate the possibility of naturally occurring phase II *C. burnetii* organisms that may cause eventually both acute and chronic infections (Williams *et al.*, 1986*c*). Situation is complicated further by identification of *C. burnetii* variants (Vodkin and Williams,

1986) that do not conform the view that phase variation is related only to LPS structure (Schramek and Mayer, 1982; Amano and Williams, 1984*b*).

One can conclude that although more concentrated effort will be necessary to solve the new problems concerning phase variation of *C. burnetii* as well as its genetic and antigenic stability, the outlook of Q-fever immunity and immunoprophylaxis seems to be more optimistic than it was 10 years ago.

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Tribute

D. Blaškovič is 75

Prof. D. Blaškovič, the founder and long years' Editor-in-Chief of Acta Virologica, member of its International Editorial Board, ordinary member of the Czechoslovak and Slovak Academies of Sciences, professor emeritus of microbiology at Comenius University in Bratislava, honorary member of several scientific institutions in Czechoslovakia and abroad, carrier of several awards — in this year, on August 3rd, achieves the age of 75 years.

The jubilee is looking back to a fruitful scientific and organisatory activity acknowledged not only in Czechoslovakia but also by the virologists in abroad. He is equally well known both among the young generation of scientists and those who laid down the foundations of basic biological research and/or the diagnostics of viral diseases after the Second World War in Europe. Some of them established lasting contacts with the jubilee when he was the director of the Institute of Virology in Bratislava, others saw him as the chairman of various scientific committees, and scientists from abroad may remember him in the function of the Editor-in-Chief of Acta Virologica. After retirement, 10 years ago, he has neither abandoned his scientific interests, nor interrupted the contacts with the Institute and the Journal he had been directing for long years. He still participates in the scientific life of our country, attends scientific conferences, works in scientific committees, follows with interest the recent developments in virology, epidemiology and molecular biology. Moreover, his recent studies on murine herpesviruses testify that prof. Blaškovič is still an active scientist sustaining the scientific contacts at home and abroad.

The International Editorial Board wishes to the jubilee good health, continuing enthusiasm and joy from the growth of the work the foundation of which hehadlaid down.

J. Rajčáni *L. Borecký*