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DIAGNOSIS AND CONTROL OF RICKETTSIAL DISEASES

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Summary. — Common isolation procedures on chick embryos and laboratory animals are not of great importance for routine diagnosis of rickettsioses. Detection of rickettsiae in skin lesions by immunofluorescence technique allows early diagnosis of Rocky Mountain spotted fever (RMSF). Broad spectrum of methods is at disposal for serological diagnosis of rickettsial diseases. Their choice is determined by laboratory equipment, profesionality of laboratory staff, economy and simplicity of the given test. Though complement-fixation and microagglutination tests held their position and will certainly be used in future, the use of indirect immunofluorescence test is recommended for its sensitivity and simplicity. Latex agglutination test is valuable especially in the diagnosis of acute rickettsial infections. Recently introduced ELISA method is expected to fulfil the highest requirements as to sensitivity in differentiation of rickettsioses within the known classification groups. The efforts to obtain efficient antirickettsial vaccines have been limited to preparation of the vaccines against RMSF and Q fever. As to the latter, elaboration of chemovaccine and preparation of chloroform-methanol-treated phase I *C. burnetii* suspension of decreased reactogenity seem promising in field trials.

Key words: laboratory diagnosis; rickettsiae; Q fever; vaccination

Introduction

In spite of the fact that rickettsial diseases still pose a serious problem world round, they have not received such public attention as many other maladies, largely because their presence or the true magnitude of their occurrence remain unrecognized. "Longevity" of rickettsial diseases as a public health problem is based on the peculiarities of ecology of their agents: rickettsiae occur in nature (at present even *Rickettsia prowazekii*, the causative agent of epidemic typhus, does not represent any exception) in arthropod vectors and animal reservoirs, in the cycles of transmission not depending on man, who, with an exception of Q fever, can be infected only occasionally by arthropod bite. Of all rickettsial diseases only epidemic typhus and Q fever may occur as extensive and explosive epidemics. Significance of the

occurrence of rickettsial diseases markedly varies in different geo graphic regions, based on the whole number of ecological factors, on the part of causative agents, and on social and economic conditions, including the level of hygiene and education, on the part of susceptible human population. Escape of man from urban areas to nature, its cultivation, the possibility of rapid migration by modern means of transport represent new factors leading to the higher frequency of cases of tick-borne rickettsioses not only in suburban areas, but also in the areas in which their occurrence has not been previously registered. However, rickettsial diseases pose the greatest problems among population groups that have the least resources for their detection, surveillance and treatment.

Clinical information, while helpful, is insufficient for identification of rickettsial disease and their reliable differentiation from other pyrexias. The clinical picture of rickettsial diseases may vary from inapparent forms to manifest ones. Q fever, because of little typical clinical signs, in acute form easily confounded with many virus and bacterial diseases, chlamydial incuding, manifests in clinically distinct chronic forms as endocarditis and hepatitis (Turck *et al.*, 1976; Turck, 1981). The possibility of application of wide-spectrum antibiotics in any febrile disease of microbial origin, weakens the efforts of exact diagnosis and leads to suppression of the development of typical clinical signs. On the other hand, lack of information on clinical picture may cause an exclusion of antibiotics from the therapy with very serious consequences, because of the high mortality in some rickettsial diseases (epidemic typhus, RMSF, scrub typhus, chronic Q fever), which were not treated with efficient antibiotics. Finally, the recently observed atypical course of some rickettsial diseases should be taken into consideration, as the lack of eschar in scrub typhus after reinfection with antigenically differing strains or the enteric fever in murine typhus cases from Kuwait and Rangoon.

Early laboratory diagnosis of rickettsial diseases

Detection of identification of *R. prowazekii* by immunofluorescence in the gut of lice collected from the body of a patient suspicious from epidemic typhus, enable to confirm diagnosis as early as within several hours after hospitalization. The haemocyte test demonstrating the presence of spotted fever group rickettsiae in ticks (Gurgdorfer, 1970; Řeháček *et al.*, 1971), 1971), provides the same possibility for early diagnosis of tick-borne rickettsioses. For early diagnosis of the latter is also at disposal the identification of rickettsiae by direct immunofluorescence in skin biopsy specimens obtained from the rash by punch biopsy, as shown in cases of RMSF (Woodward *et al.*, 1976; Walker and Cain, 1978; Walker *et al.*, 1978; Hall and Bagley, 1978; Fleisher *et al.*, 1979). Negative result of the test, however, does not exclude the possibility of rickettsial disease (Linneman, 1980). Detection of rickettisal antigens in the patient's blood and urine, as attempted in the past by many authors in epidemic cases of murine and scrub typhus, will probably be used in future at enzyme-linked immunosorbent assay (ELISA) capable to assess minimal amounts of antigen.

Routine methods of rickettsial isolation are not of a greater importance for early diagnosis, with an exception of isolation and identification of rickettsiae by primary cultivation of monocytes collected from buffy coat of patients suffering from RMSF (DeShazo *et al.*, 1976). Worth mentioning is also the biochemical method of early laboratory diagnosis of RMSF using the frequency-pulsed electron capture gas-liquid chromatographic analysis of sera to prove volatile components, which are formed as a consequence of metabolites produced by the infecting agent, as a result of metabolic changes induced in the host by the disease or as a result of specific host response (Brooke *et al.*, 1981). Even though this method is helpful from the onset ot rickettsial disease, evidently it will hardly be able to contribute to the diagnostic potential of great majority of rickettsial laboratories.

Serological diagnosis of rickettsial diseases

Serological methods still hold a leading position in diagnosis of rickettsial diseases. The spectrum of the methods used is broad, and the methods developed for other fields of microbiology are readily applied also in rickettsiology. Introduction of a new method is usually accompanied by authors commentary enumerating the disadvanteges of the older methods. Sensitivity, specificity, simplicity, economy and quickness are proclaimed as attributed of each novel method. Advances in a few top laboratories are only poorly reflected in developing countries, i.e. in regions with the highest occurrence of rickettsial diseases. The lack of specific antigens as well as of skilled workers makes it possible surviving the use of Weil-Felix (WF) reaction. In collaborative studies, the aim of which is to examine suitable sensitive and simple methods of serological diagnosis, are mostly engaged again only well equipped laboratories.

Complement-fixation (CF) and microagglutination (MA) tests

Both tests still hold and will probably hold their position and reputation in serological diagnosis of rickettsial diseases. The CF test is generally considered to become positive later in the course of disease (usually about on day 10 from the beginning of disease); it reflects the presence of specific, mostly IgG antibodies for many years, so that it is suitable for serological surveys. It is commonly used for diagnosis of all rickettsial diseases. Because as many as 5 reagents participate in this test, its results are directly proportionate to their quality, especially to the quality of rickettsial antigens, whether soluble or corpuscular. Lower susceptibility of CF test, e.g. in comparison with indirect immunofluorescence (IF) test (Newhouse *et al.*, 1979), is not its only disadvantage. Repeated assertion (in text-books and scientific papers as well) on the possibility of differentiation between epidemic and murine typhus by washed corpuscular *R. prowazekii* and *Rickettsia typhi* antigens is rather superstition than reality. The future of such a differentiation among representatives of typhus and spotted fever group rickettsiae is in the isolation and characterization of the surface protein antigens as indicated by Dasch and coworkers by demonstration of species-specific antigens in *R. prowazekii*

and *R. typhi* (Dasch, 1981; Dasch *et al.*, 1981). The possibility of such a differentiation is badly needed, because, e.g. sera of RMSF patients cross-react in CF test up to 68% with *R. prowazekii* antigen (Shepard *et al.*, 1976). Corpuscular antigens, however, were proved suitable for classification of *Rickettsia tsutsugamushi* strains in Japan, though plaque reduction assay represents the more exact approach to this problem (Oaks *et al.*, 1980).

The MA test requires highly purified antigens stained either by haematoxylin in case of *Coxiella burnetii* or by acridine orange in case of typhus or spotted fever group rickettsiae (Fiset *et al.*, 1969). Because of these requirements, the MA test is less often used than it could deserve for its reliability, sensitivity and simplicity. In Q fever it detects antibodies earlier (on days 5—6) than CF test. It can also be used for detection of residual antibodies and in serological surveys as well (Brezina *et al.*, unpublished data). For routine diagnosis of Q fever the use of artificial phase II antigen is recommended, i.e. trichloroacetic acid (TCA) — or KIO_4-treated phase I *C. burnetii* cells (Brezina und Urvölgyi, 1961; Schramek *et al.*, 1972) or phase I *C. burnetii* cells subjected to mild acid hydrolysis (Schramek *et al.*, 1978).

Both CF and MA tests are very important in diagnosis of chronic forms of Q fever, for which are significant the high levels of phase I antibodies that may reach extreme 10^5 values in either test used. However, our results (Kazár *et al.*, 1977; Brezina *et al.*, in preparation) and those of Haldane *et al.* (1983) have not confirmed the statement of Peacock *et al.* (1983) that predominance of phase I in comparison to phase II antibodies is a rule in chronic Q fever endocarditis. In both chronic Q fever endocarditis and hepatitis antibodies of IgA type can be detected by IF test.

IF test

The IF test is at present considered as the simpliest and the most economical test for early diagnosis of rickettsial diseases as well as for seroepidemiological studies and differentiation of rickettsial isolates. Microimmunofluorescence (MIF) test originally developed for diagnosis of chlamydial infections has the whole number of advantages; it requires negligible amounts of sera and antigens, serum can be titrated on one glass with several antigens, it enables determination of immunoglobulin classes and thus evidence for recent primary infection even in the case that only one serum sample is at the disposal for serological examination. The MIF test is highly sensitive and specific, its titres being higher than those of other serological reactions. The MIF test was the most consistently elaborated by Philip and coworkers for use in diagnosis of rickettsial diseases, especially of RMSF and epidemic typhus (Philip *et al.*, 1976) as well as for the typization of isolates from different tick species collected in different regions of the U.S.A. (Philip *et al.*, 1976, 1978, 1981; Philip and Casper, 1981). Due to the use of this test, the authors found 18 rickettsial serotypes, 15 belonging to spotted fever group and 3 to typhus group rickettsiae, respectively, of them 7 isolates being related with human diseases.

The IF test is widely used also in scrub typhus studies. Since 1963, when it had been first employed for serological diagnosis of the disease, it has been

used for serological classification of *R. tsutsugamushi* isolates from various animal hosts and mite vectors in different areas of scrub typhus occurrence (Shirai and Wisseman, 1975; Dohany *et al.*, 1978; Shirai *et al.*, 1980), making also possible to find out new serotypes (Shirai *et al.*, 1982). Recently it has been successfully used for serological survey of exposed army contingents in Malaysia (Brown *et al.*, 1983).

Advantages of MIF over the CF test were demonstrated in seroepidemiological survey of Q fever in some areas of Canada (Marrie *et al.*, 1984). A new qualitative solid phase fluorescent antibody test (FIAT) has recently been developed to measure antibodies in Q fever in man and animals with excellent correlation with CF results (Ascher *et al.*, 1983*a*).

In general, the MIF test was shown to be superior to the WF and CF tests. Antigens for the use in the MIF tests are purified preparations of rickettsiae killed with formalin and stabilized with merthiolate.

Indirect haemagglutination (IHA) and latex agglutination (LA) tests

It can hardly be said that the attempts of some laboratories to introduce IHA test for common diagnosis of rickettsial diseases would be proportionate to its actual use. An erythrocyte-sensitizing substance, which can be easily obtained from typhus and spotted fever group rickettsiae was characterized as to the conditions of tis extraction, resistance to trypsin and reduction of activity by sodium metaperiodate (Osterman and Eisemann, 1978). The test detects antibodies, predominantly of the IgM type, not only in humans but also in laboratory animals (Anacker *et al.*, 1979). In humans it begins to be positive from day 6 of the disease in a greater percentage than the CF test. The possibility of using glutaraldehyde-stabilized erythrocytes represents the simplification of IHA test for detection of antibodies to *Rickettsia rickettsii* in man but not in laboratory animals (Shirai *et al.*, 1975). Addition of complement changes the IHA test to passive haemolysis, in which substantially higher antibody titres than in IHA test are achieved (Bázliková *et al.*, in preparation). The IHA test can also be used for detection of phase I *C. burnetii* antibodies, when sensitizing erythrocytes by antigenic components extracted from purified phase I cells with TCA or phenol (Brezina *et al.*, 19780).

In the LA test is utilized the ability of latex particles to bind to their surface erythrocyte-sensitizing substance, so that they can be than agglutinated with antibodies directed to typhus or spotted fever group rickettsiae (Hechemy *et al.*, 1981*a*, *b*). The results of absorbtion tests showed that erythrocyte--senzitizing substances binding to erythrocytes and latex particles are not completely identical (Hechemy *et al.*, 1983*a*). Comparative study carried out in 11 laboratories revealed good correlation between the results of LA and MIF tests. The LA test was proved suitable for diagnosis of recent acute infection, providing the finding of high antibody titres, but it cannot determine immunoglobulin classes (Hechemy *et al.*, 1983*b*).

Enzyme-linked immunoassay

Recently ELISA was developed to measure rickettsial antibodies. Moreover, this sensitive, versatile test will be excellent research tool for quantitating

antibody response to antigenic fractions of rickettsiae, but the need for expensive instrumentation makes it so far impractical for routine serological diagnosis. It is more sensitive than CF test for detection of antibodies to typhus group rickettsiae, it differentiates to a certain extent between *R. prowazekii* and *R. typhi* and it is capable of detecting immunoglobulin classes. Renografin-purified rickettsial suspensions are more sensitive than particular antigens prepared by standard methods from chicken embryo yolk sacs (Halle *et al.*, 1977; Halle and Dasch, 1980). In scrub typhus, the sensitivity of ELISA corresponded to that of IF test including detection of IgM and IgG antibodies (Dasch *et al.*, 1978). In Q fever the use of ELISA for detection of IgM antibodies makes it possible in case of high antibody titre to determine correct diagnosis even when examining only one serum sample (Field *et al.*, 1983).

For diagnosis of trench fever the enzyme immunoassay using rabbit and goat antisera to guinea pig and human IgG coupled with horseradish peroxidase was tested. The results surprisingly showed antigenic relatedness of *Rochalimaea quintana* with *R. tsutsugamushi* and probably also with spotted fever group rickettsiae (Hollingdale *et al.*, 1978). Its use in studies of antigens of *R. tsutsugamushi* strains Karp, Kato and Gilliam separated by dodecyl--sulphate polyacrylamide gel electrophoresis revealed in each strain 6 antigenic components, two of which in strains Karp and Kato not reacting with heterologous sera (Eiseman and Osterman, 1981). Finally, it should be noted that Japanese authors used an direct immunoperoxidase technique for serological diagnosis of scrub typhus (Yamamoto and Minamishima, 1982).

Of importance for the choice of serological method in diagnosis of rickettsial diseases is the fact that for some tests, e.g. CF, MA and MIF, the same stock antigens can be used. Besides that, decisive is the availability of requisite equipment and supplies such as fluorescent microscopes, reliable fluorescinated conjugates, microtitre plates and dilutors.

Prevention and control

Since the IInd International Symposium on Rickettsiae and Rickettsial Diseases in 1976, in which Wisseman (1978) completely and critically summarized the state of immunoprophylaxis, chemoprophylaxis and chemotherapy of rickettsial diseases, with an exception of Q fever, only a few data have been accumulated that may lead to newer measures directed against aetiological agents, their vectors and to alteration of human susceptibility by specific immunization. This state was eventually reflected also in summarizing of the problem by WHO working group in 1982 (WHO working group on rickettsial diseases, 1982). For these reasons the analysis of contemporary state of control and prevention of rickettsial diseases will be limited only to chemoprophylaxis and vaccination.

Chemoprophylaxis

WHO working group recommends chemoprophylaxis only in special situations where adherence to a critical time-dose relationship unique for each rickettsiosis can be assured. Moreover, the ecology of scrub typhus rickettsiae

stimulates to elaboration of effective chemoprophylaxis in the problem regions. Recent results of two groups of authors classify doxycycline (Vibramicin) as an excellent antibiotic for scrub typhus prophylaxis among personel exposed to high risk of infection. Weekly dose of 200 mg protected from the disease 20 volunteers who were exposed to blood sucking by infected chiggers (Twarz *et al.*, 1982) as well as more than one thousand soldiers situated for 5 months in hyperendemic focus of scrub typhus on Pescadore Islands (Olson *et al.*, 1980). The possibility of RMSF prophylaxis was suggested by experiments in guinea pigs, in which one dose of oxytetracycline, administered shortly before expected onset of the disease, prevented its development (Kenyon *et al.*, 1978).

Vaccination

Considerable stagnation is being registered in development of vaccines, whether live of killed, against epidemic typhus. Commercially produced killed vaccines are of variable and unpredictable potency. Mason *et al.* (1976) reported the results of clinical trials evaluating four lots of typhus vaccine. One vaccine lot was selected for possible use as a federal (in the U.S.A.) reference vaccine. The future obviously lies with subunit vaccines consisting of defined and characterized specific protein antigens which appears to be nontoxic and highly immunogenic (Dash and Bourgeois, 1981; Bourgeois and Dash, 1981).

Multiple serotypes complicate the problem of preparing effective vaccines against scrub typhus. The development and duration of immunity to lethal scrub typhus infection was studied in mice vaccinated with gamma-irradiated *R. tsutsugamushi*, strain Karp. A significant difference was found in the duration of homologous and heterologous protections (Eisenberg and Osterman, 1978).

Ever lasting high incidence of RMSF in the U.S.A. stimulates interest for preparing the suitable vaccine against this disease. However, evaluation of different types of killed vaccines of rhesus and cynomolgus monkeys was not very encouraging. Cell-culture derived vaccines (chick or duck embryo cells) were proved more immunogenic than vaccines pepared from yolk sac membranes (Kenyon *et al.*, 1975*a*, *b*, 1976). Vaccine density, number of its doses and interval between doses were shown decisive for development of effective protective effect (Sammons *et al.*, 1976; Conder *et al.*, 1979). Similar results were obtained in guinea pigs in which also indicators of cell-mediated immunity were followed (Folds *et al.*, 1983). The method enabling comparison of efficiency of different vaccines in guinea pigs was elaborated (Anacker *et al.*, 1976). A new formalin-inactivated vaccine prepared by sucrose-density gradient centrifugation of tissue grown *R. rickettsii* which was evaluated in 52 persons, provided only partial protection against RMSF but ameliorated the illness (Clements *et al.*, 1983). Anacker *et al.* (1983) indicated the way to developing a subunit vaccine by isolation and characterization of surface antigens of Triton X-100 extracts of *R. rickettsii*.

No doubt, the best progress in preparation and evaluation of vaccines has

been achieved with Q fever. In contrast to other rickettsiae, phase I *C. burnetii* has a highly efficient protective antigen in its surface extractable by trichloroacetic acid (Brezina and Urvölgyi, 1961). This substance is a protein--lipopolysaccharide complex (Schramek and Brezina, 1974, 1976; Kazár *et al.*, 1978) and fulfils the requirements of subunit vaccine. It is far less toxic than the equivalent amount of phase I corpuscules from which it was extracted. It has been suggested to be promising, based on the experimental vaccination of human volunteers (Cracea *et al.*, 1973; Brezina *et al.*, 1974), though whole cell *C. burnetii* vaccine in very low doses was used recently for human vaccination (Ascher *et al.*, 1983*b*), and the possibility of removing the toxic properties for mice of phase I corpuscules by their chloroform-methanol treatment was demonstrated (Wiliams and Cantrell, 1982; Kazár *et al.*, 1983). An immunization trial carried out on 1410 persons professionally exposed to Q fever confirmed the suitability of our chemovaccine (Kazár *et al.*, 1982). Excellent evidence fo its high protective effect is the fact that it completely prevented from contracting Q fever about 150 personels or visitors of the Rickettsial Department of Institute of Virology in Bratislava, where despite of all other prophylactic measures human Q fever infections had not been unfrequent before starting vaccination. Capability of the chemovaccine to induce both humoral and cellular immune responses (Jerrells *et al.*, 1975; Kazár *et al.*, 1984) along with its low reactogenicity when excluding from vaccination persons who contracted Q fever in the past, suggest its use in personel at risk.

This review is far from completness and this has not been its purpose either. Development in rickettsiology using the favouring methods of molecular biology promises more detailed characterization of rickettsial organisms. Obligatory biotropism of rickettsiae underlines interests for the study of their physiology. There in no doubt that results from these fields will be used in improving the diagnostic methods and in preparation of efficient vaccines based on defined antigenic substances capable to induce immune mechanisms responsible for protection measurable by parameters of both humoral and cell-mediated immunity.

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