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Autor: Tringali, G.R. / Montenegro, M.R. / Walker, D.H.
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[1] Istituto d'Igiene "Giuseppe D'Alessandro", Università degli Studi di Palermo, Palermo, Italy
[2] Department of Pathology, University of North Carolina, Chapel Hill, North Carolina, USA
[3] Clinica delle Malattie Tropicali, Università degli Studi di Palermo, Palermo, Italy

Immunity and rickettsial infection: a review*

G. R. TRINGALI[1], M. R. MONTENEGRO[2], D. H. WALKER[2], S. MANSUETO[3]

Summary

Immunity to rickettsiae is enhanced by both T-lymphocytes and humoral antibodies; however, the principal effector of rickettsial killing is the macrophage. Lymphokines may play an important role. There is undoubtedly a complex in vivo interaction between the immune, phagocytic, and inflammatory host defenses against these obligate intracellular bacteria.

Key words: rickettsiae; lymphocytes; macrophages; PMNs; vaccines; lymphokines.

Introduction

Rickettsiae are small, obligate intracellular coccobacillary bacteria that reside free in the cytosol of host cells, spend all or part of their life in an arthropod host, are genetically related, and share antigens of one of the immunologic groups (typhus group, spotted fever group, and scrub typhus group). Although *Coxiella* has similarities due to its obligate intracellular parasitism, it shows considerable differences from the genus *Rickettsia* in biochemical composition, site of cellular infection (phagolysosome), developmental morphogenesis, and target cell (macrophage) in human infections (McCaul and Williams, 1981). *Rochalimaea quintana* can be cultivated on bacteriologic media. Rickettsial diseases are zoonoses in which rickettsiae are transmitted to man by the bites of infected ticks and mites (spotted fever and scrub typhus groups) or by scratching infected feces of fleas or lice into the skin (typhus group).

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Correspondence: Prof. G. R. Tringali, Università di Palermo, Istituto d'Igiene "Giuseppe D'Alessandro", Via del Vespro 133, I-90127 Palermo, Italia

From the dermal inoculation site, rickettsiae spread by the hematogenous route and penetrate vascular endothelial cells and, in Rocky Mountain spotted fever (RMSF), vascular smooth muscle cells in various organs of the body including the skin. Rickettsiae appear to induce direct injury on the parasitized blood vessels (Wolbach et al., 1922) with resulting increased vascular permeability, hypovolemia, and oedema (Harrell and Aikawa, 1949). Host defenses including inflammation, cell mediated immunity, and synthesis of specific antibodies are stimulated with concomitant killing and clearance of rickettsiae by surviving patients who subsequently, in general, have longstanding specific immunity.

In this review, we will examine the status of knowledge concerning host defense mechanisms against and immunity to rickettsiae. In the clinical setting, immune clearance of rickettsiae and recovery from illness consists of the concerted action of each of the host defenses in the previously healthy patient. As a general rule, except for epidemic typhus which often occurs in the setting of malnutrition, other infectious diseases, and harsh conditions that may stimulate the hypothalamic-pituitary-adrenal axis, the patients who contract rickettsial diseases appear to have been immunologically healthy prior to infection. Thus, experiments which are designed to dissect the components of the immune system provide data concerning immune and inflammatory mechanisms. Clearly, the interactions of these immune, phagocytic, and inflammatory mechanisms are more complex in the microenvironment of the infected vascular focus in man. Clinical judgment reveals that, in fact the complex, intact host defenses are usually quite effective. Aside from activation of the kallikrein-kinin system which might result in exacerbation of hypotension by causing vasodilation (Yamada et al., 1978), inflammation, coagulation and the immune response do not appear to act as important pathogenic mechanisms of tissue injury.

Humoral immunity

The role of humoral antibodies in immunity to rickettsiae has been known since the early passive immunity experiments of Ricketts (Ricketts and Gomez, 1908). It was subsequently shown that serum from immune guinea pigs can protect recipients from infection with *R. prowazekii* or *R. mooseri* (Zinsser et al., 1935). More recently, studies on humoral immunity following infection with *R. prowazekii* demonstrated that human immune serum conferred protection on challenge of animals and increased opsonization and destruction of rickettsiae by leukocytes and macrophages (Gambrill and Wisseman, 1973). Moreover, studies by Topping demonstrated that immune serum given to experimental guinea pigs prophylactically protected them from disease caused by *R. rickettsii* (Topping, 1940). Immune serum was also investigated as a therapeutic agent for RMSF and experimental infection of guinea pigs with *R. rickettsii* (Topping, 1943).

In vitro studies indicate that human macrophages restrict *R. mooseri* replication in the presence of antibodies but not in their absence (Gambrill and Wisseman, 1973; Beaman and Wisseman, 1976). Kekcheeva reported a good correlation between antibody titers, macrophage activation and levels of immunity (Kekcheeva et al., 1981). The presence of immune serum enhanced the activity of macrophages, thus suggesting synergistic effects of humoral and cellular immunity.

Although various species of rickettsiae have been demonstrated to contain from 27 to 30 detectable proteins of which up to six are surface proteins (*R. conorii*), the function and importance for pathogenicity and immunity is not known for any of these proteins (Obijeski et al., 1974; Tzianabos et al., 1974; Eisemann and Osterman, 1976; Osterman and Eisemann, 1978; Pedersen and Walters, 1978; Smith and Winkler, 1980). Thus, the apparent discrepancy between reports concerning the ability of antibody to prevent infection of nonprofessional phagocytes is not surprising since the content and avidity of antibodies to rickettsial proteins including possible attachment or penetration-associated proteins is not known (Wisseman et al., 1974; Turco and Winkler, 1982). Investigation of the role of antibody in protection from infection and disease awaits more complete delineation of rickettsial antigens and their roles in the physiology and pathogenic mechanisms of rickettsiae.

Cell-mediated immunity

Lymphocytes from previously infected and vaccinated humans undergo blast transformations in vitro in response to rickettsiae or their purified antigens (Coonrod and Shepard, 1971). A cell mediated immune response completely independent from humoral antibody was demonstrated by Bourgeois et al. (1980), who stimulated human peripheral blood lymphocytes with antigens extracted from renografin-purified *R. mooseri* and *R. prowazekii.* The contributions of cellular and humoral immunity in scrub typhus were investigated in inbred mice infected with several strains of *R. tsutsugamushi* (Shirai et al., 1976). Mice immunized with the less virulent Gilliam strain of *R. tsutsugamushi* were partially protected against a challenge with the Karp strain as early as the 3rd day and completely protected on day 7 after infection. Antibodies seemed not to play an important role in immunity as the antibody activity against the Karp strain was very low and no significant levels of complement fixing antibodies, a relatively insensitive indicator of antibodies, were detected early in infection. Moreover, serum from the Gilliam infected mice failed to protect recipients against the challenge with the Karp strain. In contrast, protection was afforded by passive transfer of spleen cells from Gilliam immunized animals up to the 63rd day. Passive heterologous protection was mediated by a non-adherent fraction of spleen cells, presumably T lymphocytes, since it was shown that spleen cells depleted of T lymphocytes were unable to transfer resistance

to the recipient. It should be noted, however, that homologous protection also resulted from the passive transfer of anti-Karp immune serum.

Immunity has also been successfully conferred upon non-immune guinea pigs challenged with *R. mooseri* after adoptive transfer of spleen cells collected from immune animals. Recipients were protected both from rickettsial proliferation and formation of a local lesion at the site of inoculation. In contrast, serum failed to prevent rickettsial proliferation and the formation of the inoculation site lesions (Murphy et al., 1979). It appeared that cell mediated immunity was responsible for the effective control of infection but did not completely eliminate rickettsiae. Guinea pigs which had recovered from a previous infection, however, completely and rapidly cleared inoculated microorganisms. Antibodies were detectable in the serum, and a cooperative mechanism of humoral and cellular immunity was suggested. A role for antibody in the defense mechanism of guinea pigs cannot be excluded, and at least a modulating activity can be postulated.

The importance of cell-mediated immunity against spotted fever group rickettsiae was suggested by the increased quantity of *R. rickettsii* in guinea pigs treated with immunosuppressive doses of antilymphocyte serum as compared with recipients of normal rabbit serum (Walker and Henderson, 1978). Subsequent studies by Kenyon and Pedersen showed that, although both athymic and euthymic mice produced specific antibodies to *R. akari*, the euthymic mice were able to terminate the infection with a much shorter course of antibiotic treatment (Kenyon and Pedersen, 1980). Moreover, adoptive transfer of spleen cells from immune euthymic mice conferred protection on athymic mice. In another study, adoptive transfer of immune spleen cells converted fatal *R. conorii* infection of normal and cyclophosphamide-treated inbred mice to inapparent infection and overt disease but with the majority of animals surviving, respectively (Kokorin et al., 1982). It was suggested by selective treatment of the donor cells that T-lymphocytes were the most important supplemented element.

Macrophages

Macrophages appear to be the most important final common pathway for rickettsial clearance. In human and experimental animal infections with rickettsiae, the lesions contain a preponderance of mononuclear cells morphologically compatible with macrophages and large and small lymphocytes. Nacy and Osterman (1979) have shown that both humoral and cellular responses may have a role in facilitating the destruction of *R. tsutsugamushi* by macrophages. Pretreatment of rickettsiae with antibodies resulted in a marked decrease of infection of macrophages. Furthermore, infection of macrophages by rickettsiae was markedly suppressed in macrophages treated with a preparation of lymphokines collected as supernatant of cultured spleen cells from *R. tsutsuga-*

mushi-infected mice and stimulated with heat killed rickettsiae. Soluble products released by sensitized lymphocytes in vivo or in the supernatant of stimulated cultured cells in vitro can activate resident macrophages to inhibit rickettsial infection by two mechanisms: increased resistance to infection by rickettsiae and increased intracellular killing (Nacy and Meltzer, 1979). Changes in the macrophage cell membrane during activation may cause reduction in intracellular organisms recovered from lymphokine treated macrophages by hindering rickettsial penetration. The nature of the rickettsiacidal activity was not elucidated in these studies. Although killing of intracellular parasites such as *Trypanosoma*, *Toxoplasma*, or *Listeria* is related to functional changes in the phagolysosomal system, rickettsiae escape from the phagosome into the cytoplasm. Thus, they avoid the microbicidal and degradative processes and multiply freely in the cytosol of infected cells. A cooperative role for antibodies in the uptake of rickettsiae by activated macrophages was not detectable since the phagocytic activity was poor. The studies of Nacy et al. supported the view that an early cellular response, in the absence of antibodies, was crucial in controlling the growth of *R. tsutsugamushi*. It was suggested, however, that antibodies may play a major role late in the infection when opsonization of rickettsiae augments the microbicidal activity of macrophages. However, complete protection of macrophages from successful rickettsial infection was not achieved for a few macrophages still supported rickettsial growth. Heterogeneity of the macrophage population may account for a minority population of cells which demonstrate persistence of rickettsiae regardless of the method of activation.

It is known that macrophage activation and function are under the control of genetic factors. Genetic influences have been shown to determine the susceptibility of different strains of mice to rickettsiae (Sammons et al., 1977; Groves and Osterman, 1978; Rybkina, 1981). Susceptibility to *R. akari* has been tested in several inbred and outbred strains of mice and a broad range of resistance was revealed (Anderson and Osterman, 1980). Genetic control of natural resistance to infection as well as of cytotoxic tumoricidal activity was mediated through the mechanisms of macrophage function (Meltzer and Nacy, 1980; Nacy and Meltzer, 1982; Meltzer et al., 1982). Mice with defects in tumoricidal activity may also fail to activate bactericidal activity. Mice from A strain (A/Hed) and C3H/He, which were shown to be defective in developing tumoricidal activity in vitro, were susceptible to *R. akari*. However, P/J mice which share the same tumoricidal functional defect were resistant to *R. akari*. This observation demonstrated that resistance to *R. akari* and macrophage tumoricidal activity are under control of different genes and can be dissociated. Observations on in vivo and in vitro responses of resistant Balb/c and susceptible C3H/He mice inoculated with *R. tsutsugamushi* (Gilliam strain) have shown that lack of macrophage activation in C3H/He mice was related to a defective responce to lymphokines which were produced in a normal manner. Resistance to rickettsial infection and capability to develop activated tumoricidal macro-

phages in mouse strains are controlled by different, autosomal genes. Analysis of lymphokines contained in supernatants of antigen-stimulated spleen cells allowed further definition of the biological basis of these activities. Fractionation of lymphokines by Sephadex G200 column chromatography yielded a peak of tumoricidal activity in a single fraction of molecular weight 45,000. In contrast, the activating factor for intracellular killing eluted in three different fractions of 115–125,000, 35–45,000, and $<10{,}000$ daltons, respectively (Nacy and Meltzer, 1982; Meltzer et al., 1982).

The route of inoculation, in addition to the genetic background and cellular interactions, also influences the response of mice to infection with *R. tsutsugamushi.* Certain strains of mice are resistant to intraperitoneal infection; subcutaneous inoculation produces a chronic infection with development of immunity for heterologous strains. Intravenous inoculation with the Gilliam strain of *R. tsutsugamushi* in resistant C3H/RV and susceptible C3H/He showed that both strains of mice were resistant to low dose inoculation. Modification of resistance to intraperitoneal or intravenous inoculation with *R. tsutsugamushi* after irradiation or treatment with particulate materials was suggestive of dependence of immunity on the existence of a different population of macrophages with a different sensitivity to irradiation (Jerrells and Osterman, 1982). It seems clear that macrophage activation is, therefore, the key mechanism upon which the ultimate mechanism of intracellular rickettsial killing depends, whether through the cooperativity of T cells, an antibody-dependent mechanism, or genetically determined background influences.

Avoidance of the microbicidal activity of macrophages by rickettsiae seems to occur by the escape of rickettsiae from phagosomes into the macrophage cytoplasm (Andrese and Wisseman, 1971). Thus, rickettsiae which have not been opsonized may avoid degradation following fusion of the phagosome and lysosome. Elucidation of the cellular control mechanisms of the intracellular host-parasite relationship may lead to a more complete understanding of the biology of rickettsiae.

Polymorphonuclear leukocytes

Although polymorphonuclear leukocytes have been shown to be the predominant early response to rickettsial inoculation, they have not been documented to play an important antirickettsial role. Indeed, they are present in relatively small quantities in rickettsial lesions at the crucial times in the clearance of rickettsiae. Rikihisa and Ito (1979, 1980) have confirmed the phagocytosis of rickettsiae by polymorphonuclear phagocytes of guinea pigs. Only viable intact rickettsiae were visible in phagosomal vacuoles, and it appeared that phagosomes containing viable organisms did not fuse with lysosomes, as they did with nonviable or disrupted rickettsiae. Degenerating rickettsiae were phagocytized in larger phagosomes and were enveloped by a cellular mem-

brane. Thus, viable rickettsiae appear to avoid the intracellular killing mechanisms by penetrating the phagosomal membrane to escape into the cytoplasm. Walker and Winkler (1981) documented recently that *R. prowazekii* can damage polymorphonuclear phagocytes with concomitant release of lactate dehydrogenase from the polymorphonuclear cells by a process similar to that by which they lyse erythrocytes. Cytochalasin B inhibits phagocytosis of *R. prowazekii* but has no effect on the release of LDH from polymorphonuclear leukocytes exposed to *R. prowazekii.* Thus, lytic factors such as phospholipase may damage the phagosomal membrane and allow entry into the cell cytoplasm. This finding must be evaluated further in macrophages and monocytes. Other results, however, seem to support this observation. It has been demonstrated that *R. prowazekii* may hydrolyze the phospholipids of L cells to fatty acids and lysophophatides (Winkler and Miller, 1982). These biochemical changes led to loss of integrity in the plasma membrane of cells as shown by increased permeability to trypan blue and release of LDH and ^{86}Rb. Rickettsiae inactivated by heat, ultraviolet irradiation, treatment with N-ethylmaleimide or other metabolic inhibitors did not injure L cells with release of fatty acids and lysophosphatides. Thus, the activity of phospholipase A has been related to the mechanism which allows the organisms to escape from phagosomes into the cytoplasm.

Vaccines

In clinical practice, rickettsial vaccines are rarely employed in the prevention of human disease. The history of RMSF has seen the use of two killed-rickettsia vaccines, one of tick origin (Spencer and Parker, 1930) and the other derived from yolk sacs of embryonated hen's eggs (Cox, 1939). The former was field tested and shown to have some efficacy. Both failed to protect many recipients and did not protect in challenge trials of human volunteers (Dupont et al., 1973). Another purified killed-rickettsia vaccine of cell culture origin has been developed, but also afforded incomplete protection as judged by experimental animal studies and challenge trials in human volunteers (Fiset et al., 1982; Folds et al., 1982). The specific antigens of *R. rickettsii* required to prevent infection or block the pathogenic mechanisms have yet to be identified.

A live attenuated vaccine (E strain) for epidemic typhus has been shown to be effective in a controlled field trial in Burundi and Bolivia (WHO Working Group on Rickettsial Diseases, 1982; Wisseman, 1972; Wisseman, personal communication). Subunit protein antigen vaccines for both epidemic typhus and murine typhus have been developed and have shown promising results in experimental animal models (Bourgeois and Dasch, 1981). Development of a useful vaccine against scrub typhus has been hampered by the antigenic diversity of strains of *R. tsutsugamushi.* However, a gamma-irradiated vaccine has demonstrated hopeful results in experimental animals (Eisenberg and Oster-

man, 1977). Future progress in research on rickettsial immunity and protection from disease will depend upon scientific research and the application of modern methods of cell biology to identify the rickettsial antigens, their mode of presentation to the host, and the essential components of the immune response which must be stimulated to afford longlasting protection from each rickettsial infection.

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