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ROLE OF MACROPHAGES IN INFECTION WITH *RICKETTSIA CONORII*

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Summary. — Macrophages obtained from the abdominal cavities and spleens of DBA/2 mice and guinea pigs convalescent after *Rickettsia conorii* infection digested the rickettsiae in vitro more actively than those from uninfected animals. The activation of macrophages was manifested by their capacity to inhibit replication of the rickettsiae and to digest them as well as by their resistance to the toxic effect of rickettsiae. Visual observations and bioassays showed that a portion of the rickettsial population survived in the culture producing no toxic effect on the cells which could be readily passaged.

Key words: *Rickettsia conorii*; *macrophage activation*; *immunity*

Introduction

In the past 15 years the interest in cellular factors of immunity has increased considerably owing to the great advances in non-infectious immunology and immunogenetics. Such investigations are particularly important in infections associated with obligate and facultative intracellular parasitism, especially in rickettsial diseases in which antibodies alone do not prevent infection.

In cultures of various cells, nonspecific phagocytes (chick embryo fibroblasts, yolk sac cells, etc.), rickettsiae multiply readily in the presence of immune serum (Kazár *et al.*, 1973; Wisseman *et al.*, 1974). Antibodies, however, activate phagocytosis and inhibit replication of rickettsiae in macrophages. Thus Andress and Wisseman (1971), Gambrill and Wisseman (1974), Kenyon *et al.* (1977) and Kazár *et al.* (1975) observed in cultures of macrophages from human and animal convalescents after endemic typhus, tick rickettsiosis, and Q fever increased phagocytosis of rickettsiae and inhibition of their growth only after pretreatment of rickettsiae with immune serum. On the other hand, Kekcheeva *et al.* (1978) observed more active digestion of *R. prowazekii* by macrophages derived from immune guinea pigs, and Kabanova *et al.* (1978) by macrophages derived from *R. conorii*-infected

guinea pigs as compared with macrophages from intact animals without pretreatment of rickettsiae with immune serum.

Both specific (Kishimoto and Walker, 1976; Kishimoto *et al.*, 1977) and nonspecific (Kelly, 1977) activation of macrophages derived from *Coxiella burnetii*-immunized guinea pigs was reported. The specific macrophage activation in rickettsial infections is manifested not only by a more active digestion of rickettsiae by immune macrophages (Kishimoto and Walker, 1976; Kishimoto *et al.*, 1977) but mainly by the resistance of macrophages to the toxic products of digestion (Kokorin, 1957). Polymorphonuclear leukocytes in an immune host die in the same manner as in a nonimmune one on phagocytosis of rickettsiae.

The lack of a common concept on the specific changes in macrophages in rickettsial infection appears to be due to investigations having been done in different models, with various rickettsial species, using various vaccines, methods and times of seeding the cultures, and, most important, various infective doses in the experiments. In experiments by Kekcheeva and Kokorin (1956) on the immunity in Q rickettsiosis in mice, repeated intravenous inoculation of *C. burnetii* to convalescent animals was accompanied by mass lysis of the rickettsiae by spleen macrophages 3 days after inoculation and no infection developed. In mice immunized with a killed vaccine, lysis of rickettsiae was also observed in the spleen at 3 days, but at 5 days intensive replication of rickettsiae began again and was followed by their abundant accumulation.

At present, inoculation of animals is the only reliable method for testing the intensity of postinfection and postvaccination immunity. It is therefore necessary to develop various tests of cellular immunity which could be used for immunity appraisal in man.

The aim of the present study was to investigate the role of peritoneal and spleen macrophages in the development of postinfection immunity to *R. conorii* and to compare the response to infection of macrophage cultures derived from immune and intact animals.

Materials and Methods

The experiments were carried out in cultures of macrophages derived from two animals species susceptible to *R. conorii*: guinea pigs and inbred DBA/2 mice. The animals were inoculated intraperitoneally with tissue culture *R. conorii* (M-1 strain). Cultures of peritoneal and spleen macrophages were obtained at various intervals (14—180 days) after inoculation. Mouse peritoneal macrophages were collected after injection of meat peptone broth into the abdominal cavity. The cells were cultivated in bacteriological tubes on coverslips in Eagle's minimal essential medium (MEM) with 5 % foetal calf serum. The cultures were inoculated one day after macrophage collection. Spleen macrophages were collected by the method of Mosier (1967) and inoculated 3 days after explantation.

The inoculum consisted of a suspension of rickettsiae grown in L-cell culture. A suspension of the cells filled with rickettsiae (all cells infected) was disrupted in a glass homogenizer, centrifuged for 10 min at 1000 rev/min, and diluted in 10-fold steps. The dilutions 10^{-3} — 10^{-4} in the culture medium, corresponding to 1000 and 100 ID_{50}, were used for inoculation.

Table 1. Percentage of macrophages infected with R. conorii in cultures derived from intact and convalescent mice

Mouse group	Percentage of macrophages containing rickettsial antigen			
	Total	Typical rickettsiae	Rickettsiae and antigen granules	Granular antigen and conglomerates
Intact	100	100	0	0
Infected (13 days after inoculation)	46.5	60	8.5	31.5
Intact	89.5	29.5	63.5	7
Infected (50 days after inoculation)	45.5	1	11	88

The functional activity of macrophages derived from intact and immune animals and the intensity of rickettsia multiplication in them were examined 3, 5 and 6 days after inoculation by immunofluorescence and in preparations stained according to Giemsa. Bioassays from infected macrophage cultures were done in L-cell cultures.

Results

Uninoculated macrophage cultures derived from peritoneal exudate of intact and immune mice did not differ from each other. In preparations of uninfected macrophage cultures from convalescent animals, immunofluorescence revealed no rickettsiae or rickettsial antigen beginning at 14 days after inoculation. The preparations contained well-spread stellate macrophages and round and elongated mononucleate cells with short processes and compact nucleus, frequently excentric, as well as a few mesothelial cells.

Thre days after inoculation of macrophages derived from intact mice the amount of infected macrophages in different experiments ranged from 40 % to 65 %. Most cells contained dozens and hundreds of rickettsiae showing specific fluorescence on the periphery, sometimes filling the entire cytoplasm of the cells (Fig. 1, see Plate XII). At 5 days the proportion of infected macrophages increased to 89—100 % (Table 1). Some macrophages, in addition to typical rickettsiae, contained digested rickettsiae in the form of fluorescent granules and conglomerates. The intensive replication of rickettsiae in macrophages derived from intact mice was confirmed by bioassays in L cells. At 5 days of cultivation the infectious titre of rickettsiae increased by 2 orders of magnitude. Despite intensive replication of rickettsiae their toxic effect on intact mouse cells was insignificant. The loss of macrophages in the cultures was due to great accumulation of rickettsiae and rupture of the cells filled with them.

Table 2. Percentage of macrophages infected with R. conorii in spleen cell cultures from intact and immune guinea pigs

Animals	Percentage of macrophages containing the rickettsial antigen at indicated days after inoculation					
	21	28	56	70	90	180
Intact	50±0.7	73.5±1.1	79 ±3.6	89.7±3.2	94.5±0.4	96.3±0.3
Immune	20.3±3.9	31±8.2	3.5±1.4	15.1±2.6	25.3±0.2	93.2±3.1
Index of infection	0.41	0.42	0.04	0.17	0.27	0.97

Different results were obtained in macrophage cultures derived from mice 13 and 50 days after inoculation with *R. conorii*. Five days after inoculation of the cells in culture were considerably less affected and digestion of rickettsiae in macrophages was clear-cut (Table 1). In cultures derived from the surviving mice at 50 days post infection, 45.5 % macrophages contained the antigenic material. Only 12 % of the cells contained typical rickettsiae, while 88 % macrophages only rickettsial antigen in the form of fluorescent granules and conglomerates. Bioassays in L cells and cytological examinations showed a lack of complete digestion of rickettsiae in macrophages from surviving mice. The infectious titre in the culture from immune mice remained at the initial level (100 ID_{50}) for 6 days while in cultures from the intact mice it increased by 2 orders of magnitude (10,000 ID_{50}). A more intensive digestion of rickettsiae was observed in mouse macrophages 50 days after inoculation.

The function of spleen macrophages was studied in cultures derived from guinea pigs. These cultures consisted of 2 cell types: macrophages and macrophage-like round cells and stromal fibroblast-like cells (well spread spindle-shaped and stellate cells with large nuclei, low chromatin content and 3-4 nucleoli). Groups of fibroblast-like cells appeared 3-4 days after explantation and at 6 days colonies of proliferating fibroblasts appeared.

In cultures from intact guinea pigs the rickettsiae multiplied intensively involving by 6 days from 50 % to 96 % of the macrophages (Table 2). The rickettsiae exerted a toxic effect on macrophages followed by death of many of them and release of the rickettsiae into the culture medium with intensive involvement of 32—70 % fibroblast-like cells. When subpassaged, these cultures died.

In macrophage cultures from guinea pigs inoculated 2-3 months before the removal of macrophages, the number of rickettsia-containing cells was 4-6 times lower than in cultures from intact animals, and in many cells the

passaged readily. A higher digestive activity of immune macrophages as compared with normal ones was confirmed by bioassays. The infectious titres of homogenates of cultures of infected macrophages derived from intact animals at 7 days after inoculation were by 2 orders of magnitude higher than the initial ones, while those of culture homogenates from immune guinea pigs were lower by 1 order, that is, the difference in the infectious titres of these cultures was 1000-fould.

To evaluate the digestive activity of macrophages, we calculated an index of macrophage infection (ration of the percentage of infected macrophages from immune and intact animals). As showen in Table 2, the index of infection at 2-21/2 months was the lowest, i.e. the digestive activity of macrophages was the highest. At 6 months after inoculation the digestive activity of macrophages decreased in most animals and was nearly equal to that in control animals.

Discussion

The investigations in macrophage cultures derived from the abdominal cavities and spleens of animals infected with *R. conorii* revealed specific activation of macrophages in convalescent animals. Macrophage activation was manifested by their capacity to inhibit replication of rickettsiae and to digest them as well as by a resistance to the toxic effect of rickettsiae on cells in guinea pig macrophage cultures. An increased digestive capacity of macrophages was observed from the 14th day after inoculation of the animals and the maximum activity occurred in cultures from convalescent animals at 50-70 days after inoculation. Visual observations and bioassays demonstrated that a portion of rickettsiae in the population was not destroyed and survived in the culture. But the rickettsiae exerted no toxic effect on the cells which remained viable and did not undergo degenerative changes in contrast to the cultures from intact guinea pigs. Owing to the barrier function of macrophages, stromal reticular cells were affected insignificantly, and cultures from immune animals were readily passaged despite the presence of rickettsiae. According to Wisseman *et al.* (1974), multiplication of rickettsiae in cultures of non-phagocytic cells in the presence of immune serum explains the retention of rickettsiae in cells of an immune host in nonsterile immunity.

Alongside with reports (Kokorin, 1957; Kishimoto and Walker, 1976; Kishimoto *et al.*, 1977) on the occurrence of specific macrophage activation, our investigations demonstrated macrophage activation within 1 1/2 — 3 months after inoculation of guinea pigs with *R. conorii*. Nonspecific activation of macrophages was observed for at least 2 weeks after inoculation of guinea pigs with killed *C. burnetii* (Kelly, 1977).

The mechanisms of specific macrophage activation are in the very early stages of investigation. The method of adoptive transfer has been used for transfer of cellular immunity in tsutsugamushi fever (Shirai *et al.*, 1976; Catanzaro *et al.*, 1977), in Q fever (Kazár *et al.*, 1977), and Marseilles fever (Kabanova *et al.*, 1978). The protective effect here was associated with T

lymphocytes. It is known that T cells may exert their killer function in rickettsiae showed an altered morphology and staining properties. In macrophages and stromal cells derived from immune guinea pigs necrobiotic changes were nearly absent. The number of affected stromal cells was insignificant and, unlike the cultures from intact animals, these cultures could be transplantation and antitumour immunity. In addition, they produce humoral factors and mediators by means of which they exert the effectory function and association with other cells of the lymphoid and phagocytic systems, inducing their functional activity. The specific activation of macrophages is quite likely to occur upon contact with sensitized T cells or humoral factors secreted by them. As for the mechanisms of macrophage resistance to pathogenic agents and, in particular, to rickettsiae, it may be associated with derepression of formation of some enzymes or substances inhibiting and blocking rickettsial growth in the cells. These hypotheses require further experimental confirmation.

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