

## ACTIVITY OF MACROPHAGES FROM GUINEA PIGS AT VARIOUS PERIODS AFTER INFECTION WITH *RICKETTSIA PROWAZEKI*

N. G. KEKCHEEVA, O. A. VOVK, G. E. ABROSIMOVA, E. A. CHERESHKOVA

The N. F. Gamaleya Institute of Epidemiology and Microbiology, U.S.S.R. Academy of Medical Sciences, 123 098 Moscow, U.S.S.R.

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*Summary.* — The function of peritoneal macrophages from *Rickettsia prowazeki*-infected guinea pigs at various intervals of postinfection immunity was studied. The activity of macrophages in immunized animals was higher than in non-immunized ones; it was the highest in the period of the highest level of immunity and high levels of complement-fixing antibodies.

*Key words:* *Rickettsia prowazeki*; immunity; macrophages

### Introduction

The importance of investigations on the role of cell factors, in particular macrophages, in the development of immunity in rickettsial diseases is increasing because the humoral factors, to which most attention was paid so far, do not reflect all aspects of immunological changes in the host. Under certain conditions, especially in postvaccination immunity, any correlation between antibody level and resistance to infection may be missing. The achievements of non-infectious immunology open new prospects for investigations on the role of cellular factor in infectious pathology and immunology and, in particular, in the field of rickettsial infections.

Macrophages undoubtedly play a role in resistance to infection also in typhus fever but several problems remain open. Postinfection immunity in guinea pigs is known to persist for a long time, but its intensity decreases gradually. We were interested to see whether there is a correlation between resistance to infection, activity of macrophages and level of complement-fixing (CF) antibodies. We studied the *in vitro* activity of macrophages from guinea pigs at various intervals after infection with *R. prowazeki* and compared it with the levels of immunity and CF antibody.

### Materials and Methods

Guinea pigs were infected intraperitoneally (i. p.) with  $10^4$ – $10^5$  ID<sub>50</sub> of an egg culture of the Breinl strain of *R. prowazeki*. Such infection produces a typical clinical picture: after an incubation period there follows fever and (irregularly) periorethritis. The disease is accompanied by immunological changes — development of resistance to challenge with virulent *R. prowazeki* and appear

Table 1. *In vitro* activity of macrophages from *R. prowazeki*-infected guinea pigs

Macrophages taken from guinea pigs	CF antibody titres	% of rickettsia-containing macrophages treated with normal serum			macrophages treated with immune serum		
		2 days*	5 days	7 days	2 days	5 days	7 days
13 days p.i.	320-1280	10	26	n.d.	10	13	n.d.
1 month p.i.	160-640	10	7.5	10	1	0	0
2 months p.i.	40-160	8	22	4.5	0.6	0	0
3 months p.i.	20-80	n.d.	15	n.d.	n.d.	5	n.d.
4 months p.i.	0-40	5	20	n.d.	7	11	n.d.
7 months p.i.	0-10	3.5	16.5	20	4.5	15	4
Control		3	38	21	7	16	20

\*Days of macrophage culture *in vitro*.

n.d. = not done.

ance of CF antibody in serum. The level of immunity was assayed by *i. p.* challenge with  $10^4$  ID<sub>50</sub> of a virulent culture of *R. prowazeki*. As defined by P. F. Zdrodovsky (Zdrodovsky and Golinevich, 1956) immunity is complete in guinea pigs developing no febrile reaction and periorchitis in response to challenge, partial in animals developing fever for 1-2 days, and absent in guinea pigs responding to challenge like untreated animals. The presence of rickettsiae in the animals was determined by inoculating chick embryos and guinea pigs with organ suspensions (kidneys, liver, spleen, brain).

Macrophages were collected from immune and untreated animals 3 days after *i. p.* injection of thioglycolate broth. Tubes were seeded with 500,000-600,000 macrophages in medium 199 containing 10% bovine serum. After 48 hr of cultivation in this medium the cells were infected with *R. prowazeki*. The rickettsiae were purified by differential centrifugation from yolk sacs of infected chick embryos, suspended in 20% milk, distributed in ampoules and stored at -60 °C. These preparations were used within 2-3 weeks after having determined their titres in chick embryos. Macrophage cultures were inoculated with a given dose of rickettsiae ( $10^5$ - $10^6$  ID<sub>50</sub>).

Macrophage activities were studied in the presence of normal or immune guinea pig serum. The purified rickettsiae were treated with either serum for 30 min at room temperature and inoculated into a macrophage culture. To manifest itself, the immune serum has to be added to the rickettsiae before they get into macrophages (Gambrell and Wisseman, 1973). The immune serum was obtained from guinea pigs 1½-2 months after infection with *R. prowazeki* and had a titre of 80-160; it was used in a dilution of 1:10.

The interaction of rickettsiae with macrophages was evaluated by examination of Giemsa-stained coverslips removed from the tubes at 2, 3, 5, and 7 days after inoculation (*p. i.*) of the macrophages. The activity of macrophages was estimated by their capacity to destroy phagocytized rickettsiae as judged by the number of rickettsia-containing cells. A lower percentage of rickettsia-containing macrophages was considered to be indicative of a higher digestive capacity of macrophages. The per cent of rickettsia-containing macrophages was calculated from 300-400 cells examined. At various intervals *p. i.*, the contents of the tubes were inoculated into chick embryos to establish the viability of rickettsiae and in the absence of microscopically visible rickettsiae, to check the visual observations.

## Results

Rickettsiae treated with normal serum multiplied well in macrophages from untreated animals (control). Within the first 2-3 days larger rickettsiae frequently formed filaments and chains. The per cent of rickettsia-containing macrophages was initially low (Table 1), but increased by the 5th day when the nature of rickettsiae changed. Very small forms filling the cell in tremendous numbers were prevalent. Inoculation of chick embryos with the content

**Table 2. Immunity and presence of rickettsiae in *R. prowazeki*-infected guinea pigs**

Months p.i.	CF	complete	Immunity			Rickettsiae in bioassay*
	antibody titres		partial	none	none	
1	160-640	25/27	2/27	0/27	±	
2	40-160	7/7	0/7	0/7	—	
3	20-80	3/3	5/8	0/8	—	
4	0-40	2/10	4/10	4/10	—	
7	0-10	0/9	6/9	3/9	—	

Numerator: No. of animals with the indicated degree of immunity; denominator: No of animals examined.

\*± = Rickettsiae detected in some guinea pig organs; — = rickettsiae not detected.

of the tubes at this interval showed the macrophages to contain viable rickettsiae that induced lesions in chick embryos and were revealed by microscopy in yolk sac smears. Later, by 7 days, macrophages began to die, burst and release the rickettsiae. After treatment of the rickettsiae with immune serum, the per cent of rickettsia-containing macrophages was lower than after treatment with normal serum but the rickettsiae were not completely destroyed.

A different picture was observed with immune guinea pigs, the time of examination, i.e. the period which elapsed after inoculation, being of decisive importance. The results of several experiments are summarized in Table 1. Macrophages from immune animals infected 1 month previously contained on the 5th day of cultivation much less rickettsiae than those from control animals even with normal serum (7.5 and 38 %, respectively). In the presence of immune serum the macrophages were free from rickettsiae. More active destruction of rickettsiae in macrophages in the presence of immune serum was also observed by Gambrell and Wissemann (1973), Kenyon *et al.* (1977), Nacy and Osterman (1979), and Vovk *et al.* (1980). Inoculation of chick embryos with the contents of some tube cultures which were negative on microscopic examination confirmed these observations, in that no rickettsiae were demonstrated by bioassay. But some cultures, also negative on microscopic examination, yielded very few rickettsiae in the inoculated chick embryos. Two months p. i. of the guinea pigs, the activity of macrophages was clearly demonstrated only when the rickettsiae were treated

**Table 3. Correlation of CF antibody titres and immunity in *R. prowazeki*-infected guinea pigs**

Immunity	CF antibody titres						
	640-320	160-80	40	20	10	5	0
Complete	18/19	10/10	8/8	2/6	4/10	0/8	0/7
Partial	1/19	0/10	0/8	4/6	4/10	8/8	2/7
None	0/19	0/10	0/8	0/6	2/10	0/8	5/7

Numerator: No. of animals with the indicated degree of immunity; denominator: No. of animals with the indicated CF antibody titre.

with immune serum. At later intervals (3-4-7 months) the activity of macrophages decreased gradually. Thirteen days p. i. the activity of macrophages was lower than after one month.

The results concerning immunity of the animals and detection of rickettsiae in their organs are presented in Table 2. In the first 2 months practically all animals showed "complete" immunity. Thereafter its intensity decreased, and after 3 months some animals showed "partial" and others no immunity. The highest antibody titres were found in the first month; after 2 months their level decreased gradually. Antibody persisted in low titres (5-20) for the whole period of observation (7 months). In some animals antibody was no more found at this interval. Rickettsiae were detected in the organs of some animals for up to 1 month p. i.

### *Discussion*

The present data allow the conclusion that macrophages from immune animals are more active than those from untreated guinea pigs. The highest activity manifested by destruction of the phagocytized rickettsiae was observed 1-2 months p. i. The activity was enhanced in the presence of immune serum which offers evidence of a combined effect of humoral and cellular factors of the immune host in the destruction of the agent. Three months p. i. the activity of macrophages was slightly lower than at 1 and 2 months, at 4 and 7 months p. i. it decreased further. A comparison of these data with the results of immunity testing in guinea pigs by inoculation with a virulent culture showed the period of the highest macrophage activity to correlate with the period of the highest level of immunity in guinea pigs (see Table 2). In this period (1-2 months p. i.) practically all animals (32 out of 34) had complete immunity, i. e. challenge with a virulent culture did not result in the development of clinically manifest disease. The same period (1-2 months p. i.) was characterized by a high titre of CF antibody (as a rule, at least 80). The highest antibody levels were found 2-3 weeks p. i., i. e. slightly earlier than the highest macrophage activity, but the antibodies persisted at a sufficiently high level for 1½-2 months. A comparison of the antibody level and intensity of immunity showed a certain correlation between these values (Table 3). Animals with antibody titres of 40 or higher showed complete immunity on challenge with virulent rickettsiae. At antibody titres lower than 40 both complete and partial immunity occurred. As mentioned above, antibody in titres of 40 or higher were found, as a rule, up to 3 months p. i., i. e. in the period of most intensive immunity and highest macrophage activity. Thus, there was a certain correlation between the level of immunity, macrophage activity, and antibody level, the highest macrophage activity having been observed in the period of the highest intensity of immunity. Naturally, the factors under study cannot fully correspond to each other because the immunity of the host is determined not only by the function of macrophages but also by other factors. Nevertheless, the activity of macrophages reflects not only immunological changes as such but to a great extent also the degree of these changes.

A comparison of the levels of antibody and macrophage activity with the finding of rickettsiae in the host makes it possible to conclude that the highest level of these immunological indices were typical of a stage during which the agent was still found in the host (up to 1 month) and then for another  $\frac{1}{2}$ -1 month.

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