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COMPARATIVE STUDY OF ROSETTE- AND PLAQUE-FORMATION IN RATS INFECTED WITH *Mycoplasma arthritidis* AND *Acholeplasma laidlawii*

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Mycoplasma arthritidis was shown to inhibit rosette and plaque formation in rats infected with this species of mycoplasma. By the 15th day the immune response in the control and experimental groups was equal again. At later stages strong stimulation of populations of rosette-forming (RFC) plaque-forming (PFC) cells was observed, subsiding toward the 150th day. Conversely, *Acholeplasma laidlawii* stimulates RFC and PFC at all periods of infection. The relatinship between these phenomena and the pathogenic properties of mycoplasmas is discussed.

KEY WORDS: mycoplasma; rosette formation; plaque formation.

There is evidence that cellular immunity plays an essential role in protection against infection caused by mycoplasmas (arthritis, acute respiratory diseases, etc.) [4, 11]. The study of cellular reactions is therefore important in connection with the study of mechanisms of development of the lesions.

The dynamics of the primary immune response to sheep's red blood cells was investigated in animals infected with *Mycoplasma arthritidis* and *A. laidlawii*.

These species of mycoplasmas were chosen because one of them (*M. arthritidis*) is a generally accepted pathogenic species, giving rise to polyarthritis in mice and rats, whereas the other (*A. laidlawii*) belongs to a genus whose pathogenicity for rats has not been proved.

EXPERIMENTAL METHOD

All the experiments were carried out on Wistar rats weighing 140-250 g. *M. arthritidis* (strain PG6) and *A. laidlawii* were grown at 37°C in broth prepared from a tryptic digest of bovine heart with the addition of 20% normal horse serum. The animals were infected intraperitoneally with undiluted 4-day cultures of *M. arthritidis* [dose 10^8 colony-forming units (cfu) in 1 ml] and 2-day cultures of *A. laidlawii* (dose 10^9 cfj/ml), and also with cultures diluted 10 and 100 times with broth. Control animals were given an injection of 1 ml broth.

Immunization with sheep's red blood cells was carried out 5 days before the immune response was tested. The population of rosette-forming cells (RFC) was studied by the method of Biozzi et al. [3] in the modification of Khorobrykh et al. [1], and the population of plaque-forming cells (PFC) by the method of Jerne and Nordin [6].

EXPERIMENTAL RESULTS

During the first week after infection with *M. arthritidis* the immune response to sheep's red cells was sharply inhibited in the experimental animals compared with that in the control (Fig. 1). The degree of inhibition was a linear function of the dose of the mycoplasmas. After infection with the undiluted culture the number of RFC and PFC was reduced by 83 and 90% respectively compared with the control. A dose of 10^7 cfu/ml inhibited rosette and plaque

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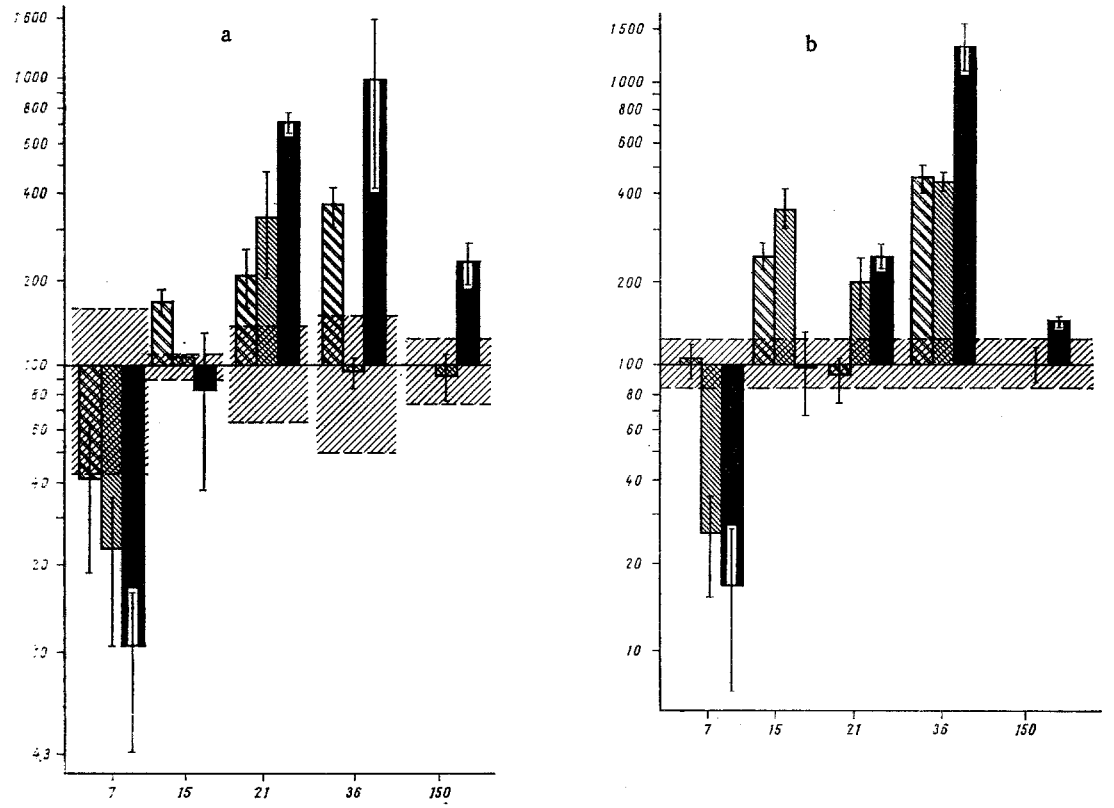

Fig. 1. Rosette formation (b) and plaque formation (a) in rats infected with *M. arthritidis*. Abscissa, time after beginning of infection (in days); ordinate, number of rosettes per 10^3 cells and number of plaques per 10^6 cells as percentages of corresponding values in control for animals infected with *M. arthritidis* in a dose of 10^8 cfu/ml (black columns), 10^7 cfu/ml (thickly shaded columns), and 10^6 cfu/ml (thinly shaded). Vertical lines and shaded region represent standard deviation in experiment and control.

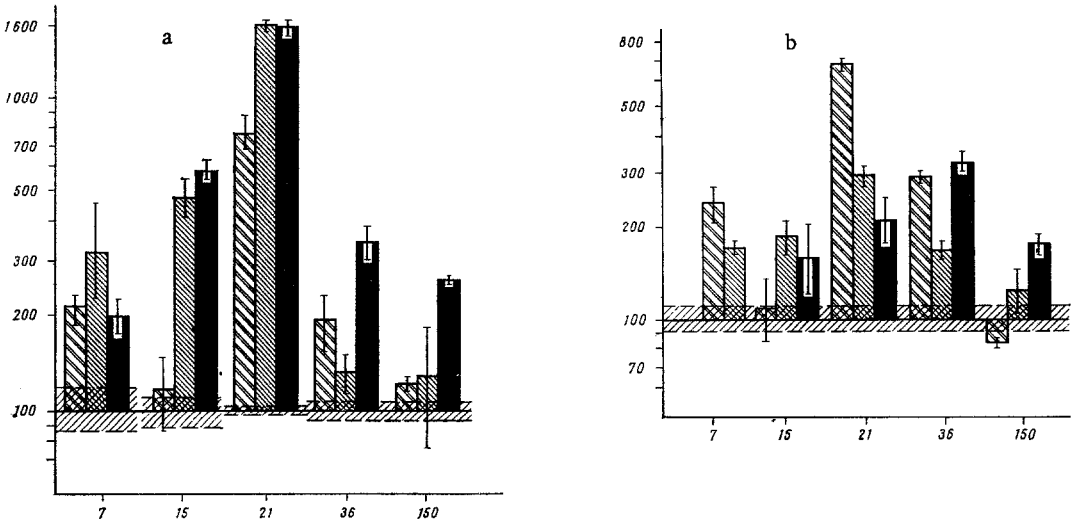

Fig. 2. Rosette formation (b) and plaque formation (a) in rats infected with *A. laidlawii*. Legend as in Fig. 1. Black columns correspond to dose of 10^9 cfu/ml, thickly shaded 10^8 cfu/ml, thinly shaded 10^7 cfu/ml.

formation by 74 and 73%. Cultures diluted 100 times or more did not cause any significant change in the number of RFC and PFC.

During the next 7 days the inhibition diminished and by the end of the 2nd week of infection the primary immune response was practically the same in the experimental and control

groups. Later inhibition was replaced by stimulation. Initially this process took place particularly quickly for RFC in animals infected with diluted cultures: On the 15th day of infection the number of RFC was 240 and 349% respectively of the control for doses of 10^6 and 10^7 cfu/ml. Later, however, these indices increased more slowly in these groups than in rats infected with the undiluted culture, in which the number of RFC 21 and 36 days after infection was 237 and 1280% respectively, and the number of PFC 701 and 990% respectively. Having reached a maximum on the 21st-36th day, the immune response returned slowly to normal, but even 150 days after infection some increase in production of RFC and PFC (140 and 224%) could still be observed in animals infected with the maximal dose of *M. arthritidis*.

Infection of rats with *M. arthritidis* thus leads to inhibition of rosette and plaque formation during the first 2 weeks after infection, but this is followed by stimulation, and the immune response does not return to normal until the 150th day.

The lymphoid system of rats reacts completely differently to infection with *A. laidlawii* (Fig. 2). In this case inhibition of the immune response was not observed. On the contrary, toward the end of the 1st week of infection, the number of PFC was 81-106% greater than in the control animals. The largest number of PFC in rats infected with *A. laidlawii* was observed, just as in the experiments with *M. arthritidis*, on the 21st day after infection. A particularly strong effect (about 1600%) was found when *A. Laidlawii* was given in doses of 10^9 and 10^8 cfu/ml, but later it decreased appreciably and by the end of the 5th month of infection significant stimulation of PFC (261%) could be observed only in the group of animals infected with a dose of 10^9 cfu/ml (Fig. 2a).

The RFC population was stimulated to a lesser degree by *A. laidlawii*. Dose dependence in this case also was ill-defined (Fig. 2b). Nevertheless, here also stimulation reached maximal values on the 21st-36th days, and by the 150th day it was visible only in the group of animals infected with the undiluted culture of *A. laidlawii*.

A. laidlawii thus stimulates rosette and plaque formation at all stages of infection, and differs significantly in its action from *M. arthritidis*.

The differences described above can be connected with the ability of *M. arthritidis*, unlike *A. laidlawii*, to utilize arginine. The action of mycoplasmas on the immune system of the host is known to be largely connected with this property [10]. Mycoplasmas which utilize arginine have two opposite actions on the lymphoid system: inhibition on the one hand, and stimulation on the other. The first of these effect predominates over the second when mycoplasmas are used in sufficiently high doses (over 10^6 cfu/ml in experiments in vitro) and it is connected with the arginine-deiminase system [10]. The stimulating factor has not been identified [5].

Since *M. arthritidis* utilizes arginine, it can tentatively be suggested that inhibition of rosette and plaque formation caused by this mycoplasma in the early stages of infection is connected with the circulation of an adequate number of microorganisms during this period. With a decrease in the concentration of mycoplasmas, inhibition is replaced by stimulation. Stimilation may be due to the antigen of the mycoplasmas [10], which persists for a particularly long time in the spleen [7].

Mycoplasmas not utilizing arginine, including *A. laidlawii*, are in many cases polyclonal stimulators of lymphocytes, and the intensity of this stimulation is comparable with that due to phytohemagglutinin and lipopolysaccharide [2, 8, 9].

The fact that *A. laidlawii* has no arginine-deiminase system is one possible reason for the absence of inhibition of RFC and PFC during the first 2 weeks of infection. On the other hand, activation of lymphocytes caused by different species of mycoplasmas may play an essential role in infection caused by mycoplasmas [5]. However, as has already been stated, *M. arthritidis* differs in its pathogenicity from *A. laidlawii*; for that reason, in the investigation of the mechanisms of pathogenesis it is particularly important to make a combined study of the immunologic picture and to discover the causes not only of the similarity, but also of the difference between the different species of mycoplasmas.

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