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IMMUNOLOGIC REACTIVITY OF (C57BL/6 × A/Sn)F_1 MICE IN MIXED MYCOPLASMA-VIRUS INFECTION

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Mixed infection of hybrid mice, highly resistant to Rauscher virus, with this virus and Mycoplasma arthritidis was accompanied by progressive inhibition of populations of splenic rosette-forming (RFC) and plaque-forming (PFC) cells and led to induction of malignant erythroblastosis, cytologically identical with Rauscher's leukemia. During mixed infection of the hybrid mice with Acholeplasma laidlawii and Rauscher virus the immune response was almost completely suppressed on the 21st day and considerable splenomegaly was observed, but by the 62nd day of infection the RFC and PFC populations and also the weight of the spleens had regained the control level. The possible role of mycoplasmas in the induction and development of Rauscher's leukemia is discussed.

KEY WORDS: Mycoplasma arthritidis; Acholeplasma laidlawii; Rauscher leukemia virus; immune response.

Mixed infection of (C57BL/6×A/Sn)F_1 mice highly resistant to Rauscher virus with this virus and Mycoplasma arthritidis leads to induction of transplantable malignant erythroblastosis, cytologically identical with Rauscher leukemia in sensitive mice [2]. Similar infection of mice of the same line with Rauscher virus and Acholeplasma laidlawii is accompanied by short-lasting splenomegaly followed by regression and does not lead to leukemia [3], as does mixed infection with the virus and mycoplasmas. An abundance of data has been obtained to show that these agents persist in mixed infection [1-3], but there is virtually no information on immunologic reactivity.

The object of this investigation was to study the effect of mixed mycoplasma-virus infection on splenic rosette-forming (RFC) and plaque-forming (PFC) cells and on serum hemagglutinin titers in infected mice.

EXPERIMENTAL METHODS

(C57BL/6×A/Sn)F_1 mice aged 6-8 weeks were used. The mycoplasmas and virus were obtained as described previously [2, 3].

The mice were infected intraperitoneally with M. arthritidis with a titer of 10^8 colony-forming units (CFU)/ml or with A. laidlawii with a titer of 10^9 CFU/ml, in a dose of 0.5 ml, and with Rauscher virus in a titer of 10^5 ID_{50}/ml in a dose of 0.1 ml. Control mice were inoculated with broth in which the mycoplasmas were grown.

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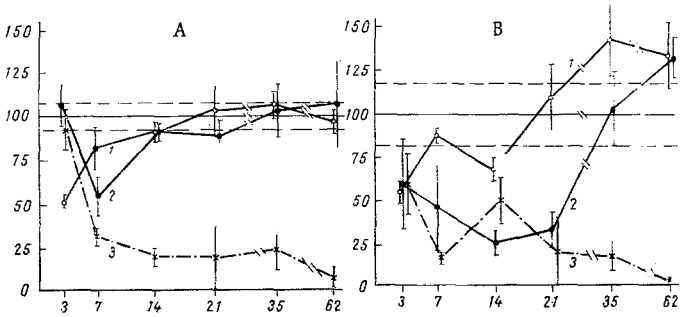

Fig. 1. Rosette- and plaque-formation in mixed mycoplasma–virus infection. 1) Infection with M. arthritidis; 2) infection with Rauscher virus; 3) mixed infection with mycoplasma and virus. Ordinate, number of RFC per 10^3 and number of PFC per 10^6 nucleated cells, expressed as a percentage of control. Abscissa, time after infection, in days.

TABLE 1. Effect of Mixed Mycoplasma–Virus Infection on Weight of the Spleen and Synthesis of HA against Sheep's Red Blood Cells in (C57BL/6 × A/Sn)F_1 Mice (M ± m)

Infection	Index	Days of infection				
		3rd	7th	21st	34th	62nd
C	Total HA titer	3,61±0	3,51±0,28	3,81±0,37	3,91±0,38	3,16±0,45
	Weight of spleen	166±27	127,3±11,2	166,7±18	161,3±27	154±14
M	Total HA titer	2,11±0,3	3,76±0,45	3,01±0,3	3,46±0,15	2,71±0,6
	Weight of spleen	121,3±7,8	178±69	206±37,5	239,5±75,5	193±5
A	Total HA titer	3,01±0,3	3,61±0,6	3,76±0,75	3,61±0,6	3,01±0,6
	Weight of spleen	193±42	142,5±23,5	182,5±4,5	170,5±21,5	155,5±7,5
V	Total HA titer	2,71±0,6	3,51±0,51	3,11±0,38	3,61±0,45	2,71±0,45
	Weight of spleen	154±32	214±53,7	257,3±34,7	184±56,3	228±40
M + V	Total HA titer	3,01±0,38	3,46±0,45	2,71±0,6	2,41±0,9	1,21±0
	Weight of spleen	142±30,4	249±42	1362±1138	1493±1127	3020±2164
A + V	Total HA titer	3,01±0,37	3,61±0,6	2,71±0,6	3,31±0,3	3,16±0,45
	Weight of spleen	243,5±47,5	468,5±2,5	1874±1446	730±410	254,5±124,5

Legend. C) Control, M) M. arthritidis, A) A. laidlawii, V) Rauscher virus. HA titers shown in log values, weight of spleen in milligrams.

The immunologic reactivity of the mice was determined 1, 3, 7, 14, 21, 34, and 62 days after infection, and the mice were immunized with sheep's red blood cells (in a dose of $5 \cdot 10^8$ cells) each time 5 days before the experiment.

The number of RFC was determined by the method of Biozzi et al. [5] in the modification of Khorobrykh et al. [4]. A lymphocyte to which five or more red blood cells were attached was taken as a rosette.

PFC were detected by the method of Jerne and Nordin [7]. The total hemagglutinin (HA) titer against sheep's red blood cells was determined in inactivated (30 min, 56°C) mouse serum and the titer of 7S HA was determined after preincubation of the sera with 0.2M mercaptoethanol (40 min, 20°C).

EXPERIMENTAL RESULTS

In the experiments of series I the effect of M. arthritidis and Rauscher leukemia virus on rosette- and antibody-forming activity of the mouse lymphocytes was studied.

Mycoplasmas inhibited the RFC population by 48% on the 3rd day of infection (Fig. 1a) but had no effect on the number of RFC at subsequent times. Rauscher virus caused no change (except on the 7th day) in the RFC population. During mixed infection, as early as on the 7th day considerable suppression of RFC was observed, to reach 92% on the 62nd day, after which the mice began to die from leukemia. Mycoplasmas had similar action on PFC in the spleen, and stimulation was observed only in the later stages (Fig. 1b). Rauscher virus inhibited the PFC population until the 21st day of infection, after which the immune response returned to or even above normal. Conversely, mixed infection inhibited plaque formation at all times, and on the 62nd day the PFC population was reduced by 99%.

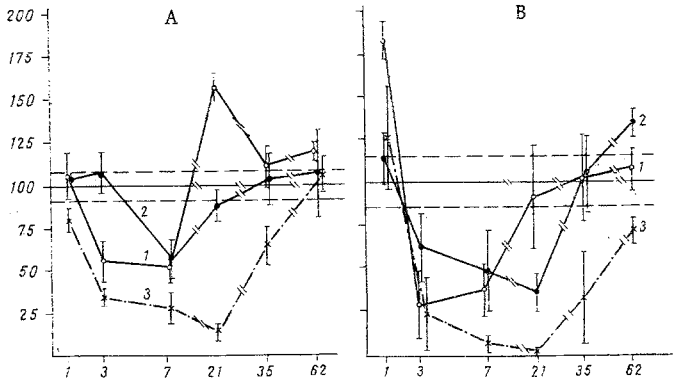

Fig. 2. Rosette- (A) and plaque-formation (B) in mixed Acholeplasma–virus infection: 1) infection with A. laidlawii; 2) infection with Rauscher virus; 3) mixed Acholeplasma–virus infection. Ordinate, number of RFC per 10^3 (A) and number of PFC per 10^6 (B) nucleated cells, in % of control. Abscissa, time after infection, in days.

Changes in the HA titers on the whole followed a similar course to changes in the PFC population (Table 1). Starting with the 21st day after infection production of 19S and 7S HA began to fall sharply in mice with mixed infection, and on the 62nd day 7S HA were virtually absent. Splenomegaly (Table 1) was found on the 7th day in mice with mixed infection, and later the weight of the spleen rose steadily.

Mixed Acholeplasma–virus infection differed in principle from the above. A. laidlawii had an inhibitory action on rosette- and plaque-formation on the 3rd and 7th days after infection, after which the immune response returned to normal (Fig. 2). Conversely, during mixed infection suppression of the immune response reached a maximum on the 21st day: the RFC population was reduced by 85%, virtually no PFC were present, and the maximal weight of the spleen reached 3.3 g. Later these indices were quickly restored and by the 62nd day they had almost reached the control level. Not a single case of leukemia developed.

Extracts of M. arthritidis and A. laidlawii are known to stimulate Friend's leukemia virus in sensitive mice [6, 11]. Similar stimulation also has been observed after inoculation of mice with erythropoietin [12] and bacterial endotoxin [10]. The authors cited suggest that the stimulating effect is the result of ability of the agents to induce immunodepression or to promote an increase in the number of target cells for the virus.

In the present experiments A. laidlawii inhibited the immune response on the 3rd and 7th days of infection, whereas M. arthritidis did so only on the 3rd day. Rauscher virus inhibited plaque formation in the early stages and stimulated it in the late stages, as was recently demonstrated in the case of resistant mice [9]. Meanwhile only mixed mycoplasma–virus infection leads to the development of leukemia [2], whereas mixed Acholeplasma–virus infection ends with complete recovery of the mice. This makes any direct connection unlikely between immunodepression induced by both mycoplasmas in the early stages of infection and the power to induce leukemia. Induction of malignant erythroblastosis in mixed mycoplasma–virus infection of resistant hybrid mice may perhaps take place as a result of the overcoming ot the natural resistance of the mice to Rauscher virus, verified at the level of target cells for this virus [8]. The subsequent development of leukemia is evidently closely linked with progressive immunodepression induced by mixed mycoplasma–virus infection.

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ANALYSIS OF A POPULATION OF Bacillus subtilis L-FORMS IN A FICOLL DENSITY GRADIENT

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The distribution of cells of a population of L-forms of Bacillus subtilis during isopycnic centrifugation in a Ficoll density gradient was analyzed. Two basic functions of the L-forms differing from one another in their buoyant density were distinguished. A study of the fractions by various methods showed that a high proportion of the population of L-forms consists of nonviable cells of different sizes, containing fragments of the genome or not containing DNA.

KEY WORDS: L-forms; density gradient; genome.

The results of phase-contrast and also of transmission and scanning electron microscopy have shown the well-marked polymorphism of L-forms [5, 6]. Members of the L-population differ not only in size, but also in submicroscopic structure and also, possibly, in methods of reproduction [1]. For this reason, separation of L-forms into fractions differing in biological properties is very interesting. The most widely used method for fractionation of L-forms, by filtration under pressure through filters with different pore diameters [9], yields fractions which differ only in the size of their L-forms. However, in this case the fractions are not even homogeneous in size. For instance, the absence of a rigid cell wall of the L-forms and their ability to undergo plastic deformation readily suggests that during filtration through pores, cells much larger than the diameter of the pores in the filters used are able to pass through [11].

In the investigation described below the L-population was analyzed by a method of separation based on buoyant density in a Ficoll density gradient.

EXPERIMENTAL METHODS

When the gradient material was chosen consideration was paid to the fact that it must not be toxic for L-forms, and the gradient produced must ensure isotonicity throughout its extent. This last property was essential so that the density of the cells would not change during centrifugation as they entered zones with different osmotic pressures of the medium. These requirements are satisfied by Ficoll-400 (from Pharmacia, Sweden), which has been used previously to study mycoplasmas [2, 3]. Separation in a Ficoll density gradient was carried out for different types of L-forms of B. subtilis, which were described as unstable, conditionally stable, and stable L-forms [4], in a Beckman L-65 centrifuge with SW50 rotor at 4°C for 5 h at 40,000 rpm. The NADH-oxidase activity of the separated fractions was determined by Pollack's method [8] and compared with the number of colony-forming units (CFU) and the DNA content (in cpm) in each fraction. For the electron-microscopic study of the fractions the generally accepted method of fixation, that of Ryter and Kellenberger, was used. The cells were dehydrated in alcohols and acetone and embedded in Araldite. The material was examined in the JEM-100B microscope with an accelerating voltage of 80 kV and instrumental magnification of 30,000 and 10,000×.

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