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ENDOGENOUS COLONY FORMATION IN MICE UNDER THE INFLUENCE OF *Mycoplasma arthritidis* AND RAUSCHER LEUKEMIA VIRUS

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Mycoplasma arthritidis was shown not to affect endogenous colony formation in lethally irradiated BALB/c mice. The number of endogenous foci in mice irradiated in sublethal doses and infected with *M. arthritidis* was sharply increased when the mycoplasma was injected 24 h before or 4 h after irradiation. Mixed infection of $(C57BL/6 \times A/Sn)F_1$ mice resistant to Rauscher virus with this virus and mycoplasma led to a marked increase in the number of endogenous colonies in the early stages of infection. This phenomenon may perhaps lie at the basis of the loss of resistance in the hybrid mice and the appearance of leukemia in them as a result of mixed infection with mycoplasma and virus.

KEY WORDS: mycoplasma; Rauscher virus; endogenous colonies.

Mycoplasmas are generally accepted as the agents of various diseases of man and animals. The role of mycoplasmas has been demonstrated in certain autoimmune disorders and in leukemias. For instance, infection of $(C57BL/6 \times A/Sn)F_1$ mice, resistant to Rauscher virus, with that virus and *Mycoplasma arthritidis* leads to malignant erythroleukemia, whereas infection with the mycoplasma or the virus alone does not induce leukemia [1, 2]. The mechanism of induction of leukemia in resistant mice during mixed infection with mycoplasma and virus is not yet clear. The possibility cannot be ruled out that interaction between mycoplasmas and hematopoietic stem cells (HSC), which are the target cells for Rauscher virus, plays a special role in the development of this phenomenon.

The object of the present investigation was to study the effect of *M. arthritidis* and Rauscher virus on endogenous colony formation in mice.

EXPERIMENTAL METHODS

Mice of lines BALB/c, $(C57BL/6 \times A/Sn)F_1$, and C57BL/6 were used. The mycoplasmas and virus were obtained as described previously [1]. Cloning of hematopoietic cells was carried out in vivo in lethally irradiated mice by the method of Till and McCulloch [11]. BALB/c mice were irradiated in a dose of 750 rad, but the $(C57BL/6 \times A/Sn)F_1$ mice were irradiated in a dose of 920 rad; 4 h later each recipient received an intravenous injection of 0.5 ml of a suspension containing syngeneic bone marrow ($5 \cdot 10^4$) or spleen ($5 \cdot 10^5$) cells. The recipients were infected intraperitoneally with mycoplasmas (0.5 ml) at the same time; the maximal titer was 10^8 colony-forming units (CFU)/ml. Irradiated mice injected with medium 199 only or with

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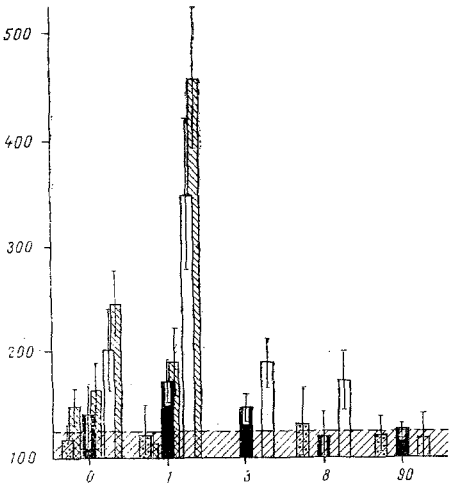

Fig. 1. Effect of M. arthritidis on endogenous colony formation in BALB/c mice. Abscissa, time after infection until irradiation (in days). On day 0 infection carried out 4 h after irradiation; ordinate, number of endocolonies (in % of control) in mice infected with mycoplasmas in doses of 10^8 CFU/ml (unshaded columns), 10^7 CFU/ml (black columns) and 10^6 CFU/ml (dotted columns). Obliquely shaded columns represent numbers of colonies in mice irradiated in a dose of 550 rad, remaining columns in a dose of 620 rad.

mycoplasmas only served as the control. Animals used for the study of endocolonization were irradiated in a dose of 550 or 620 rad. Infection was carried out 4 h after irradiation or 1, 3, 8, and 90 days before irradiation. The infecting dose was 0.5 ml of mycoplasmas and 0.1 ml of plasma containing Rauscher virus in a titer of 10^4 ID_{50} in 0.1 ml. On the ninth day after irradiation the mice were killed, their spleens were fixed in Bouin's solution, and macroscopically visible colonies were counted.

EXPERIMENTAL RESULTS

In the experiments of series I the effect of M. arthritidis on exogenous colony formation was studied in lethally irradiated mice receiving injections of bone marrow or spleen cells. None of the doses of mycoplasmas used had any significant effect on the number of colonies. Incubation of bone marrow cells with mycoplasmas in vitro (30 min, 37°C) likewise did not affect the number of exogenous colonies formed. However, the number of colonies in lethally irradiated control animals after injection of mycoplasmas differed significantly from the number of colonies in mice receiving medium 199 only: 0.90 ± 0.33 and 0.15 ± 0.15 in BALB/c mice and 1.6 ± 0.50 and 0 in the hybrid mice respectively.

The activating effect of mycoplasmas on endogenous foci was the basis for the next series of experiments. Injection of mycoplasmas into BALB/c mice irradiated in sublethal doses stimulated the formation of endogenous foci 4 h after irradiation (Fig. 1, day 0); the effect was a linear function of the dose of mycoplasmas. If infection was carried out 24 h before irradiation, the stimulating effect rose sharply and, in mice infected with the maximal dose of mycoplasmas, it reached 460% compared with the control. In mice infected three and eight days between irradiation the effect gradually diminished and then finally disappeared.

The effect of M. arthritidis and Rauscher virus on endogenous colony formation in mice resistant to Rauscher virus was studied in series II. Neither mixed infection with mycoplasmas and virus nor infection with mycoplasmas and virus separately had any effect on the number of endogenous foci in the C57BL/6 mice. After analogous infection of $(C57BL/6 \times A/Sn)F_1$ hybrid mice, infection 4 h after irradiation (Fig. 2, day 0) and 1 day and, in particular, three days before irradiation sharply enhanced endogenous colony formation. The colonies formed in mice infected with mycoplasmas were large, rather blurred in outline, and showed a ten-

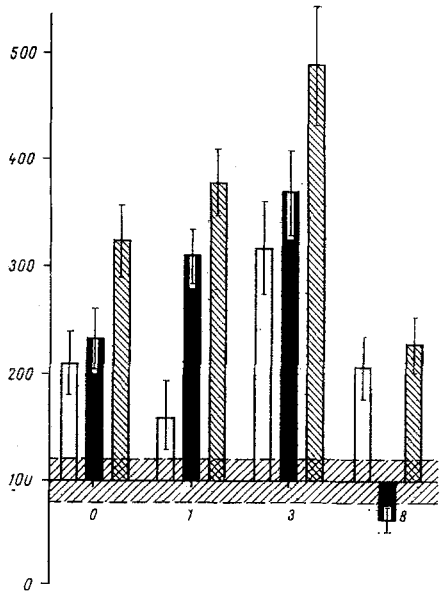

Fig. 2. Effect of M. arthritidis and Rauscher virus on endocolony formation in (C57BL/6 × A/Sn)F_1 mice irradiated in a dose of 620 rad. Abscissa, time after infection until irradiation (in days). On day 0 infection carried out 4 h after irradiation; ordinate, number of endocolonies (in % of control), in mice infected with Rauscher virus (unshaded columns), M. arthritidis (black columns), and a combination of virus and mycoplasma (obliquely shaded columns). Number of colonies on eighth day in spleens of mice infected with virus and after mixed infection was determined after counting two or three spleens in each group, for in the remaining spleens growth of the colonies was confluent.

dency toward confluent growth at all times except the eighth day. After virus infection the colonies were usually small, resembling nodules, and confluent growth was observed in a few cases only on the eighth day, when the mean weight of the spleens was 3.5 times greater than in the control. After mixed infection colonies of both types were observed, but confluent growth was characteristic, especially on the eighth day, when scarcely any individual colonies could be distinguished against the background of the sharp increase in size and weight (sixfold) of the spleens.

Bacterial endotoxins and vaccines, heterologous sera and plasma, antilymphocytic serum, and other agents are known to stimulate endogenous colony formation in mice when injected in vivo [3-6, 12]. It is considered that the stimulating effects may perhaps be connected with increased migration of HSC from the bone marrow into the spleen [3, 4, 6, 12]. In the present experiments stimulation of endocolonization in BALB/c mice infected with M. arthritidis four days after irradiation and, in particular, 24 h before irradiation, and the sharp decrease in the number of colonies on the third day of infection, suggest that under the influence of the mycoplasmas "recruiting" of HSC may take place in the early stages of infection, with a subsequent increase in recirculation of HSC and an increased inflow of them into the spleen. The absence of discharge of cells from the bone marrow on the third and subsequent days of infection may be connected with termination of the action of the mycoplasmas on HSC. The possibility cannot be ruled out that, under the influence of infection, the radioresistance of the mice was increased as a result of survival of a large number of HSC, as has been shown in the case of Rauscher virus after infection of sensitive mice [7].

The fact that larger colonies were formed by infection of hybrid mice with M. arthritidis compared with the colonies induced by Rauscher virus can perhaps be explained by the action of the mycoplasma and virus on

different precursors. The target for Rauscher virus is perhaps a more differentiated cell than the pluripotent stem cell, for example,it may be cells committed to the erythroid series [8, 9, 13]. If, however, the mycoplasma can promote erythrodifferentiation of the HSC at the expense of the other branches of hematopoiesis, this must contribute to the appearance of a larger number of target cells for Rauscher virus, and since these cells are at a later stage of differentiation than HSC, they can pass through fewer mitoses and must therefore form smaller colonies. It has been shown that certain endotoxins, if injected simultaneously with Friend virus into mice resistant to this virus, reduce their resistance as a result of a probable increase in the number of target cells for the virus [10]. It may be that the mycoplasmas act in a similar way in mixed infection of the (C57BL/6 × A/Sn)F_1 mice resistant to Rauscher virus in the present experiments. This could explain the effect of stimulation of endocolony formation during mixed infection compared with the two forms of monoinfection (Fig. 2). It was perhaps on account of an increase in the number of target cells for Rauscher virus that resistance to it was abolished in (C57BL/6 × A/Sn)F_1 mice infected with both M. arthritidis and Rauscher virus. It is interesting to note that in C57BL/6 mice, in which, as our experiments show, similar mixed infection does not lead to the development of leukemia, combined infection with mycoplasma and virus likewise does not affect endogenous colony formation.

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