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Effect of Silica on the Pathogenic Distinction Between Herpes Simplex Virus Type 1 and 2 Hepatitis in Mice

S. C. MOGENSEN* AND H. KERZEL ANDERSEN
Institute of Medical Microbiology, University of Aarhus, 8000 Aarhus C, Denmark

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The role of macrophages in the difference in liver pathogenicity between herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in mice was investigated by selectively blocking the macrophage function of the mice by silica. Intravenous administration of 3 mg of silica 2 h before virus inoculation partially abolished the difference between the two virus types, as judged by macroscopic and microscopic examination of the livers and by virus isolation studies. Intraperitoneal inoculation of 50 mg of silica before virus seemed more effective in suppressing the macrophage function, since this treatment almost completely eliminated the difference in hepatotropism between HSV-1 and HSV-2 as assessed by the number and size of the lesions appearing in the liver. The final outcome of the infection, death from encephalitis, was, however, not influenced by macrophage blockade.

In a previous study it was shown that a specific pathogenic distinction exists between herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infection in mice (11). On intraperitoneal (i.p.) inoculation with strains of HSV-2, most mice develop progressive focal necrotizing hepatitis, whereas HSV-1 only occasionally produces a few tiny self-limiting foci of necrosis in the liver.

Recently, the role of macrophages in this difference in pathogenicity between the two closely related virus types was examined, and results were presented which indicated that macrophages might play a crucial part in the phenomenon, as it was shown that the restriction of HSV-1 replication in peritoneal macrophages from mice was much greater than that of HSV-2 replication (10).

Silica, an agent reported to be selectively toxic for macrophages (2, 6), has been used in several studies to delineate the role of macrophages in the host defense mechanisms against a variety of virus infections (3-5, 7, 12-16). The purpose of the present study was to add further evidence to the role of macrophages in the pathogenic action of HSV-1 and HSV-2 in mice by selectively blocking the macrophage cell population by administration of silica.

MATERIALS AND METHODS

Mice. Four-week-old female inbred specific-path ogen-free mice of the BALB/c/A/BOM strain were used. They were obtained from Gl. Bomholtgaard Laboratory Animal Breeding and Research Center, Ry, Denmark.

Viruses. HSV strains MacIntyre (HSV-1) and MS (HSV-2), used in this study, were described previously (11).

Silica. Silica in the form of Dörentrup Quartz no. 12 (<5 μm) was kindly provided by J. Lindenmann, Institut für Medizinische Mikrobiologie der Universität Zürich. It originates from A. C. Allison, Clinical Research Center, Harrow, Middlesex, England, and has been found active for macrophage blockade in his hands (J. Lindenmann, personal communication). Dry, autoclaved silica was suspended in phosphate-buffered saline just before use at a concentration of 15 mg/ml for intravenous (i.v.) and 50 mg/ml for i.p. injection. Immediately before injection it was dispersed by brief exposure to ultrasonic vibration.

In vivo experiments. Silica was injected slowly in the tail vein (3 mg/0.2 ml) of 4-week-old BALB/c mice. Two hours after the silica injection, the mice were inoculated i.p. with 10^5 plaque-forming units of either HSV-1 or HSV-2 in 0.1 ml of diluent. Groups of control mice received HSV-1 or HSV-2 only. On the following days, five mice in each group were exsanguinated under light ether anesthesia, and the liver was examined for macroscopic lesions. Morbidity and mortality control groups of 10 mice receiving silica alone, HSV alone, or silica plus HSV were observed at daily intervals for illness and death. In the i.p.-treated group, silica (50 mg/1.0 ml) was also administered 2 h before virus.

Assay of organs for virus. At daily intervals, livers, spleens, and brains were collected aseptically from the five exsanguinated mice. Pools of five organs were frozen at −70°C until homogenized to a 10% suspension in Eagle minimum essential medium supplemented with 10% calf serum and antibiotics. After centrifugation at 4,000 × g for 30 min, the supernatants were titrated in human embryonic lung cell cultures by a plaque method previously described (9).

Histology. At autopsy, liver specimens were removed from mice of each of the groups and fixed in 4% formalin. The fixed livers were embedded in paraffin, and histological sections were prepared and stained with hematoxylin-eosin at the Institute of Anatomy, University of Aarhus.

RESULTS

Influence of silica on the development of liver lesions in HSV-1- and HSV-2-infected mice. Groups of 4-week-old BALB/c mice were injected i.v. with 3 mg of silica before i.p. inoculation with 10^5 plaque-forming units of HSV-1 or HSV-2, and five mice in each group were killed the next days. In control groups receiving HSV-1 or HSV-2 only, the previously described patterns (11) of liver involvement were seen. In brief, HSV-2-infected mice revealed focal necrotic foci in the liver, appearing on day 3 as small white spots that increased in size during the next few days. HSV-1-inoculated animals, on the other hand, showed only a few tiny lesions on the liver margins in a few cases on days 3 and 4. These lesions apparently heal, as no lesions have ever been seen when studying mice later in the course of infection.

Silica pretreatment by the i.v. route fundamentally changed this clear distinction between HSV-1- and HSV-2-infected mice. In both groups of silica-treated mice, lesions were macroscopically visible as early as 24 h after virus inoculation as discrete, tiny spots distributed all over the liver surface. During the next few days the size of the lesions gradually increased in both groups. The HSV-2 foci progressed to confluence in some mice on day 6, whereas the HSV-1 lesions, after day 2, expanded much more slowly and reached their maximum size on day 4, after which no further growth occurred. Furthermore, there were more lesions in the silica-pretreated groups than in the control HSV-2 group.

In an experiment using i.p. inoculation of 50 mg of silica 2 h before virus inoculation, the animals were killed 4 days after infection. In this experiment, the difference between the size and number of HSV-1- and HSV-2-induced lesions was almost completely abolished (Fig. 1).

Influence of silica on virus growth in liver, spleen, and brain. Figure 2 shows the titers of virus obtained in livers, spleens, and brains of HSV-1- and HSV-2-inoculated mice with and without i.v. silica pretreatment. Noteworthy in the untreated groups are the lack of virus in both liver and spleen of HSV-1-infected animals on days 5 to 7 and the fact that virus was grown from HSV-2-infected mice until death. This difference was partially abolished by silica pretreatment. During the course of infection, the

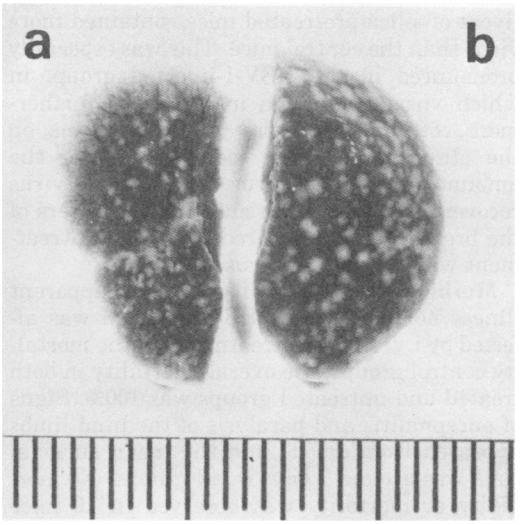

FIG. 1. *Livers from 4-week-old BALB/c mice injected with silica (50 mg/ml, i.p.) 2 h before i.p. inoculation of 10^5 plaque-forming units of HSV-1 (a) or HSV-2 (b). The mice were killed after 4 days (×2.5).*

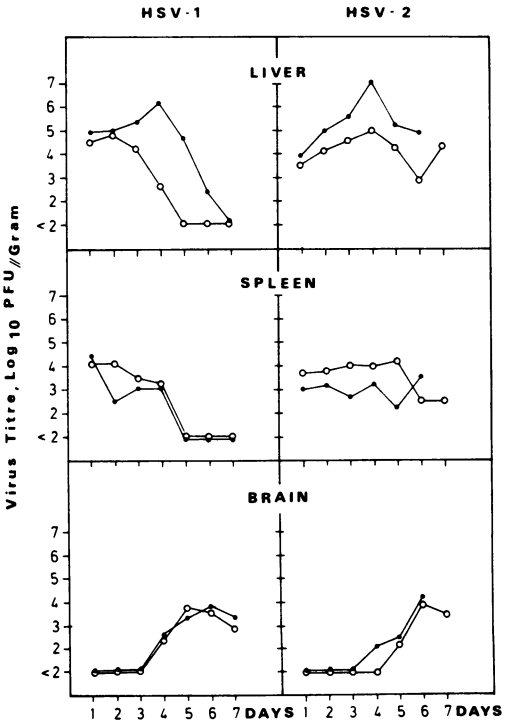

FIG. 2. *Titers of virus in livers, spleens, and brains of 4-week-old BALB/c mice with (●) and without (○) silica pretreatment (3 mg/0.2 ml, i.v.) 2 h before i.p. inoculation of 10^5 plaque-forming units (PFU) of HSV-1 or HSV-2. Each point represents a pool of five organs.*

livers of silica-pretreated mice contained more virus than the control mice. This was especially pronounced in the HSV-1-infected group, in which virus production in the liver, furthermore, continued until day 6. In the spleens, on the other hand, silica seemed to lower the amount of recovered virus. The time for virus recovery from the brain and the virus titers of the brains were not altered by i.v. silica treatment with any of the viruses.

Morbidity and mortality. Neither apparent illness nor death from HSV infection was affected by i.v. silica pretreatment. In the mortality control groups, the overall mortality in both treated and untreated groups was 100%. Signs of encephalitis and paralysis of the hind limbs appeared from day 6, and the mean survival time was around 7 days in all groups. No morbidity or mortality was observed in 10 mice treated only with silica i.v.

Histology. Lesions were seen in liver sections from all virus-infected animals, but not from any of the mice receiving i.v. silica only. Microscopically, the lesions were disseminated in the liver, with no relationship to the architecture of the lobule.

The sequences of morphological events produced by HSV-1 and HSV-2 with and without i.v. silica pretreatment were fundamentally alike, but the liver lesions differed very much in their time course, number, and size (Table 1). The main histological features were focal areas of degenerating liver cells, which were soon infiltrated with polymorphonuclear leukocytes causing central necrosis. This event was apparently not able to terminate the infection, since acutely degenerating liver cells were still present in the periphery of the lesions during this phase. Later, the necrotic lesions were infiltrated with macrophages and angioblasts, and after restoration of circulation the infection was finally arrested more or less successfully.

In the untreated control groups, the HSV-2 lesions were by far most pronounced, and although extensive infiltration of macrophages was established, the lesions were difficult to terminate (see Table 1). Treatment with silica caused a 20-fold increase in the number of lesions of both HSV-1- and HSV-2-infected animals as compared with the respective control groups, and the size of individual foci of necrosis increased two to three times. Furthermore, the regenerative events were retarded for about 1 day in both groups, which allowed the lesions to progress further.

DISCUSSION

Silica has been used as a selective macrophage-toxic agent since the early 1960s in many animal experiments to evaluate the role of macrophages in the host response to virus infections. With most viruses examined, silica has been found to potentiate the invasion and spread of the infection to target organs (3, 7, 12–16). In a few instances, silica treatment has, however, failed to break down resistance to infection (4) or has even enhanced the resistance (5).

In a previous study (10), results have been presented which indicate that macrophages play a decisive role in accounting for the difference in the ability of HSV-1 and HSV-2 to induce macroscopically visible necrotic lesions in the liver of i.p.-inoculated mice (10). The present study adds further evidence to the participation of macrophages in the difference in host response against these two closely related virus types, since it was possible to abolish the difference in hepatotropism by selectively blocking the macrophage function of the animals by the administration of silica.

In the i.v.-treated groups, macrophage blockade seemed to be effective only for a few days since HSV-1 and HSV-2 lesions progressed

TABLE 1. *Time course of inflammatory lesions in the liver of i.v. silica-treated[a] and control mice inoculated i.p. with 10^5 plaque-forming units of HSV-1 or HSV-2*

Group	Lesions		Liver cell degeneration on day:						Polymorphonuclear infiltration and necrosis on day:						Infiltration with macrophages and capillaries on day:					
	No.[b]	Maximum size[c] (μm)	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
HSV-1	1	200	(+)[d]	(+)	+	−	−	−	−	+	+	−	−	−	−	+	++	++	++	+
Silica + HSV-1	20	600	++	++	++	+	−	−	+	++	++	−	−	−	−	−	+	++	++	++
HSV-2	2	1,000	+	++	++	++	+	−	−	++	++	+	+	−	−	+	++	++	++	++
Silica + HSV-2	40	1,500	++	++	++	++	++	+	+	++	++	++	++	+	−	−	+	++	++	++

[a] 3 mg/0.2 ml 2 h before virus.
[b] Average number of lesions per 20-mm^2 section.
[c] The lesions grew in diameter as long as liver cell degeneration in the periphery progressed.
[d] Grading of the type of lesion referred to in the title: −, absent; (+), sparsely represented; +, present; ++, extensively represented.

equally well during this period, whereas a relative retardation of the HSV-1 lesions were seen from day 3 onward and no further growth of lesions occurred after day 4. Also, in the HSV-2-infected group, silica pretreatment caused a more severe infection, which from the study of the histological events and virus yield seemed to be very difficult for the animal to control; however, the lesions seemed to be terminable, as the regenerative changes also dominated these livers in the last days of the study (Table 1). The final outcome of the liver infection is, however, difficult to evaluate, since the animals were ill and died of encephalitis from day 6 onward.

In the i.p.-treated group the size and number of HSV-1 and HSV-2 lesions on day 4 were quite comparable, indicating a more prolonged macrophage ablation. The reason for this could be the 17-fold-higher (50 versus 3 mg) silica dose used in the latter groups in connection with a probably more continuous absorption of the silica.

Microscopically, the morphologies of HSV-1- and HSV-2-induced liver lesions with and without silica pretreatment were fundamentally alike. Although liver lesions were detectable in sections of livers from HSV-1-infected control mice throughout the infection, these lesions were generally so discrete that they were not visible to the naked eye. In the liver, the macrophage serves as a functional barrier to virus penetration (8), and blockade of this barrier in the initial steps of the infection allowed a more plentiful establishment of liver necrosis to take place. Furthermore, a more unrestricted progression of the lesions was seen because the invasion of macrophages was retarded.

Silica administered i.v. did not influence the final outcome of infections with HSV-1 and HSV-2 as measured by the overall mortality and mean survival time; neither did it increase the virus titers obtained in the brains of mice. Zisman et al. (15) found an increased mortality and earlier death from HSV-1 infection after silica administration. However, they used the apparently more effective i.p. route of administration of silica together with a 10-times-lower virus dose better suited to reveal the importance of a peripheral barrier to virus spread to the central nervous system. When the barrier has been overcome, be it due to macrophage blockade or a high virus inoculum, the infection probably runs its own course in the central nervous system because macrophages seem less important in this organ (1).

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