Comparative Lung Pathology of Inbred Strains of Mice Resistant and Susceptible to Sendai Virus Infection

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ABSTRACT. BALB/cA, DBA/2N and CDF1 (BALB/cA x DBA/2N) mice were inoculated intranasally with the Mol strain of Sendai virus (SV), and their mortality and histopathological lung lesions were compared. BALB/cA and CDF1 were resistant and DBA/2N was susceptible in terms of mortality. The lung lesions of the resistant strains were mild and focal, and limited to the bronchial regions, whereas those of the susceptible strain were severe and diffuse, extending to the alveoli. SV antigen was found mainly in the bronchial epithelium in the resistant strains, but in the susceptible strain, the antigen was found also in many alveolar epithelial cells and alveolar macrophages. SV antigen was detected in neither regenerated bronchial epithelium nor endothelial cells. Tumor necrosis factor (TNF) was detected immunohistologically in edematous perivascular regions and in some mononuclear cells infiltrating to the regions, suggesting that TNF is involved in the development of lung lesions by SV infection in the three mouse strains.—KEY WORDS: inbred mouse, lung lesions, Sendai virus, susceptibility.

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Sendai virus (SV) is a serious respiratory pathogen in mice and rats. Different susceptibilities of inbred mouse strains against SV infection have been confirmed by many investigators [5–7, 12, 22, 23, 26, 30]. The median lethal doses (LD₅₀) of SV in the resistant and susceptible strains differed 10⁴–10⁵ fold [12, 22]. In many viral infections, mortality was generally correlated to the degree of viral multiplication in target organs [3, 8, 9, 15–19, 21, 24, 25]. In SV infections, however, the degree of multiplication of the virus in both resistant and susceptible mouse lungs was shown to be similar [12, 22].

To clarify the mechanism of strain differences in susceptibility to SV, histopathological changes and the distribution of viral antigen after SV infection in the lungs of both resistant BALB/cA and susceptible DBA/2N mice were examined. Since SV is an inducer of tumor necrosis factor (TNF) in human lymphoid cells [1, 4] and some early changes in SV-infected mice, e.g., apparent body weight loss and neutrophil infiltration and edema in the lungs [2, 11] seems to coincide with the biological effects of TNF [10, 27], we also examined TNF together with the viral antigen immunohistologically in the lung lesion in these two strains of mouse.

MATERIALS AND METHODS

Mice: Seven- to 8-week-old female BALB/cAJcl, DBA/2NJcl and Jcl: CDF1 (hybrid of BALB/cA and DBA/2N) mice (CLEA Japan, Tokyo) which had been confirmed free from SV, mouse hepatitis virus, mouse adenovirus, mouse poxvirus, *Mycoplasma pulmonis* and *Pasteurella pneumotropica* infections [14] were used. Mice were housed in autoclaved polycarbonate cages with wood shavings as bedding and given γ ray-irradiated pellets (CE-2, CLEA Japan, Tokyo) and autoclaved tap water *ad libitum*. The animal room was controlled at 22 to 28°C, 50 to 60% relative humidity and a 12 hr light/dark cycle.

SV: The Mol strain [29] of SV propagated in the allantoic cavity of 10-day-old chick embryos was used. The infected allantoic fluid diluted to 10⁻⁵ with Eagle's minimum essential medium (Nissui, Tokyo) was used as inoculum. The virus titer in the inoculum was 1 × 10⁵ median tissue culture infective doses (TCID₅₀)/ml in LLC-MK2 cells. Mice were inoculated intranasally with 20 μ l of the inoculum under ether anesthesia. Three mice each were sacrificed on days 3, 5, 7, 10 and 14 postinoculation (p.i.) by ether anesthesia and bleeding. Lungs, thymus and spleen were taken for histopathology.

Histological and immunohistological examination: The tissues were fixed in buffered formalin and embedded in Paraplast® (Monoject, U.S.A.). Sec-

tions were made and stained with hematoxylin and eosin. Additional sections were stained immunohistologically using the Vectastain® ABC kit (Vector Laboratories, Inc., U.S.A.) for detection of SV and TNF antigens. Anti-SV rabbit serum was prepared by hyperimmunization with the purified MN strain of SV. Anti-mouse TNF- α rabbit serum was purchased from a commercial supplier (Genzyme, U.S.A.). These antisera were used at 1:500 dilution with phosphate buffered saline (PBS, pH 7.4).

Numerical estimation of severity of lung lesions was performed as follows. For the bronchial lesion, the number of bronchi and bronchioles with SV antigen positive cells and/or inflammatory cell infiltration on the sections from five lobes was counted. For the alveolar lesion, SV antigen positive cells were counted in 10 microscopic fields (x400) on each section from five lobes.

RESULTS

Fifteen mice from each three strains. DBA/2N, BALB/cA and CDF1, were inoculated intranasally with 2×10^3 TCID₅₀ of SV. Six DBA/2N, two BALB/cA and one CDF1 died 6 to 10 days p.i. Lung consolidation and thymic atrophy were recognized grossly in these dead mice of the three strains. The incidences of lung consolidation on days 3, 5, 7 and 10 p.i. were 1/3, 2/3, 3/3 and 1/1 in DBA/2N; 0/3, 2/3, 2/3 and 3/3 in BALB/cA; and 0/3, 2/3, 1/3 and 2/3 in CDF1, respectively. The gross lung lesions were severer in DBA/2N than in BALB/cA and CDF1 on days 5, 7 and 10 p.i.

Histopathologically lung lesions developed in the three mouse strains, but the degree of the lesions in DBA/2N mice was severer than those in others. On days 3 and 5 p.i., degeneration of the bronchial epithelium, migration of a few neutrophils into the bronchi, cellular debris in the bronchial lumens, mild to moderate macrophage and neutrophil infiltration in the alveoli and mild to moderate perivascular edema and hemorrhage with mild cell infiltration were observed (Fig. 1). The degenerated bronchial epithelium showed loss of cilia, irregularity in size and arrangement, giant cell formation and desquamation. Mild necrotic lesions in the alveolar septa were seen only in DBA/2N. The lung lesions on day 5 were severer than on day 3 in the three strains. On day 5 p.i., many denuded bronchi and bronchioles with severe perivascular edema and inflammatory cell infiltration were seen. On day 7

p.i., regeneration of bronchial epithelium and perivascular mononuclear cell infiltration appeared (Fig. 2). The regenerated epithelium was basophilic and hyperplastic. On day 10 p.i., regeneration of bronchial epithelium and perivascular lymphoid cell accumulation were predominant and cuboidal or squamous metaplastic changes appeared in the epithelium of the alveolar ducts and of alveoli (Fig. 3). On day 14 p.i., the lung lesions were similar to those on day 10, while only surviving BALB/cA and CDF1 were examined.

SV antigen was detected in all the strains of mice on days 3, 5, and 7 p.i. Many SV antigen positive cells were detected especially on day 5 p.i. (Fig. 4).

The antigen was seen in the cytoplasm of bronchial and bronchiolar epithelium including giant cells, alveolar epithelium, alveolar macrophages and cellular debris and neutrophils in the bronchial lumens, but not in regenerated bronchial epithelium, endothelial cells and inflammatory cells infiltrating into perivascular spaces in any of the specimens. SV antigen was also detected in the nucleoli of some bronchial epithelial cells which were positive for the antigen in the cytoplasm.

On day 3 and 5 p.i., TNF antigen was detected in edematous perivascular spaces and in cells infiltrating into the spaces of the three mouse strains (Fig. 5). There was no mouse strain-dependent difference.

Severity of the infection in the lung of the three mouse strains was estimated quantitatively. The percentage of SV-infected bronchi and bronchioles was higher in DBA/2N than in BALB/cA and CDF1 on days 3 and 7 p.i. (Fig. 6). The number of SV antigen positive cells in the alveoli was obviously much higher in DBA/2N than in BALB/cA and CDF1 on days 3 and 7 p.i. (Fig. 7). Lung lesions in DBA/2N were diffuse and extensive, whereas those in BALB/cA and CDF1 were focal and strictly limited to the involved bronchial regions (Fig. 8).

Severe cortical atrophy in the thymus was seen on days 5, 7 and 10 in the three mouse strains. No remarkable changes were seen in the spleen. No SV and TNF antigens were detected in the thymus and spleen.

DISCUSSION

Except for T cell deficient mice [13] the course of SV infection is acute and transient in mice. Although infection is not always lethal, death

Fig. 1. Early lung lesion with bronchitis, perivascular edema and mild alveolitis. DBA/2N mouse. Day 3 p. i. HE stain. $\times 40$.

Fig. 3. Cuboidal or squamous metaplasia of the epithelium of alveolar ducts. BALB/cA mouse. Day 10 p. i. HE stain. $\times 200$

Fig. 5. TNF antigen in perivascular spaces. DBA/2N mouse. Day 5 p. i. Immunohistological staining by ABC method. $\times 200$.

Fig. 2. Regeneration of the bronchial epithelium. BALB/cA mouse. Day 7 p. i. HE stain. $\times 100$.

Fig. 4. Sendai virus antigen positive cells in the bronchial epithelia and alveolar spaces. DBA/2N mouse. Day 5 p. i. Immunohistological staining by ABC method. $\times 100$.

Fig. 8. Comparison of lung lesions in BALB/cA (right) and DBA/2N (left) mice. Day 5 p. i. HE stain. $\times 100$.

Fig. 6. Percentages of Sendai virus infected bronchi and bronchioles. (○) BALB/cA, (●) DBA/2N, (△) Hybrid of BALB/cA and DBA/2N.

Fig. 7. Number of Sendai virus antigen positive cells in alveoli: total of 10 microscopic fields ($\times 400$). (○) BALB/cA, (●) DBA/2N, (△) Hybrid of BALB/cA and DBA/2N.

occurred usually on 6 to 10 days p.i. probably due to dyspnea caused by viral propagation and interference of lung circulation following the inflammatory process. We [12] reported previously that mortality was remarkably different after SV infection between resistant BALB/cA and susceptible DBA/2N, as reported by other investigators [22], although virus titers in the lung and serum antibody titers were similar in the two strains. In this study, CDF1 mice, F1 of the two strains, were shown to be a resistant phenotype similar to that of BALB/cA because of low mortality and mild and focal lung lesions. The results suggest a dominant genetic trait of the resistance. Parker *et al.* [22] reported that the

variation in sensitivity to SV was not the result of a genetic restriction on virus infection and replication but rather was the result of a physiological factor(s) possibly related to some aberrations or differences in the humoral or cell-mediated immune response. Brownstein [7] noted that the polymorphic loci regulating lung virus titers contributed to the resistance/susceptibility phenotypes.

The lung lesions in resistant BALB/cA and CDF1 were focal and limited to the bronchial regions whereas those in susceptible DBA/2N were diffuse and spreading to the alveoli. In the resistant strains, secreting antibody, cytotoxic T cells and interferon produced locally in the lungs might block the spread of SV from the bronchi to the alveoli. Brownstein *et al.* [6] reported almost the same serum interferon levels in resistant and susceptible mouse strains with SV infections.

In the present study, TNF was detected only in the edematous perivascular region where no SV antigen-bearing cells were detected, and there was no differences between the mouse strains. Nevertheless, since excessive TNF was reported to be lethal to the hosts [20, 28], susceptibility to TNF should be considered as a factor in the strain differences in susceptibility to SV-infection.

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