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Histopathologic Changes of the Spleen in Suckling Rats Inoculated with Hantaan Virus

Hye Je Cho[1], M.D., Luck Ju Paek[2], M.D., Ho Wang Lee[2], M.D.

[1]Department of Pathology, Sanggye Paik Hospital, College of Medicine, Inje University
[2]Department of Microbiology, College of Medicine, Korea University, Seoul, Korea

The purpose of this study is to delineate the histopathologic findings of the spleen after Hantaan viral inoculation, which is the largest lymphoid organ in rats, and to identify the viral location by anti-Hantaan virus (HTNV) monoclonal antibody.

All the sixty one suckling rats of less than twenty four hours of age were used. Except twenty one rats of control group, twenty-five rats inoculated intracerebrally for the early change and fifteen suckling rats inoculated intramuscularly for the late change were uniformly susceptible to lethal infection with the ROK 84-105-1 strain of seed HTNV.

The characteristic histopathologic findings were; appearance of macrophages below the splenic capsule on the 3rd day, small lymphocytes around the periarteriolar sheath on the 5th day increasing in numbers on the 7th day, and a markedly expanded marginal zone with some immunoblasts and plasma cells as well as decreased extramedullary hematopoiesis on the 9th and 14th days.

Time of onset of histopathologic changes in spleen thickness, appearance of medium and large lymphocytes and degree of extramedullary hematopoiesis were influenced by inoculation route, whereas expansion of the marginal zone was affected by postnatal age.

Key Words: *Hemorrhagic fever with renal syndrome, Hantaan virus, suckling rats, spleen, histopathologic findings.*

INTRODUCTION

Hantaan virus (HTNV), named after the Hantaan river which is located in the northern part of South Korea, is an etiologic agent of hemorrhagic fever with renal syndrome (HFRS). The agent was not only identified in the striped field mouse, Apodemus agrarius (Lee & Lee, 1976), but also isolated from patients (Lee et al, 1978). Recent epidemiologic surveys have shown that Hantaviruses are widely distributed throughout the world. A close etiologic relationship has been established among Korean hemorrhagic fever (KHF), epidemic hemorrhagic fever (EHF) in Japan and China, nephropathia epidemica in Scanndinavia and HFRS in the USSR (Lee et al, 1978; Lee et al, 1979a; Lee et al 1979b; Lee et al, 1980).

Therefore, the World Health Organization Working Group recommended in 1982 that all the diseases be referred to as hemorrhagic fever with renal syndrome (Lee, 1982).

Recently, newborn outbred suckling mice were shown to be susceptible to the HTNV infection and an immune-mediated mechanism was suspected in the pathogenesis (Tsai et al, 1982; Kurata et al, 1983; Kim et al, 1985).

We tried to delineate the histopathologic findings of the spleen after HTNV inoculation, which is the largest lymphoid organ in rats, and to identify the viral location by anti-Hantaan virus monoclonal antibody.

Address for correspondence: *Hye Je Cho, Sanggye Paik Hospital, College of Medicine, Inje University, #761-1, Sanggye-Dong, Nowon-Ku, Seoul, 139-207, Korea Tel) 938-0100 X2636*

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MATERIALS AND METHODS

1. Animal, virus dose and inoculation route

Sixty one Sprague-Dawley suckling rats of less than twenty four hours of age were obtained from pregnant female rats without serum antibodies for HTNV. Among them, twenty five suckling rats (5 rats for each period) were inoculated with ROK 84-105 strain of Hantaan seed virus intracerebrally and fifteen suckling rats (5 rats for each period) intramuscularly. The viral inoculum to the intracerebral route was 0.02 ml of $10^{9.5}$/ml LD_{50} HTNV and that to the intramuscular route was 0.1 ml of $10^{9.5}$/ml LD_{50}. The remaining twenty one suckling rats (3 rats for each period) were used as a control group. Then, one group inoculated intracerebrally (ICG) were sacrificed on the 1st, 3rd, 5th, 7th and 9th days along with the corresponding control rats and the other group inoculated intramuscularly (IMG) were sacrified on the 7th, 14th and 17th days along with the corresponding control groups.

2. Histopathologic studies

One-half of each spleen was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin, and periodic acid-Schiff. For a sequential comparison for the period, the mean values of the spleen thickness, the ratio of white to red pulp, the number of rats showing distinct marginal zone around the periarteriolar sheath and the degree of extramedullary hematopoiesis were measured in five rats of each experimental group on the 1st, 3rd, 5th, 7th & 9th days in the ICG and on the 7th, 14th and 17th days in the IMG, and three rats of each control group.

3. Immunofluorescent studies

The remaining half of each spleen was frozen and stored at −60°C until use. Sections (4 um each) were cut in a cryostat, dried, and fixed in absolute acetone in 10 minutes and stained by indirect method. The primary antibody was anti-ROK 84-105-1 viral rat antiserum at 1:16 dilution and the secondary antibody was FITC labeled goat anti-rat IgG (Miles Co.) at 1:8 dilution.

4. Immunohistochemical studies

Two kinds of monoclonal antibodies for HTNV were used. One was obtained from the Catholic University Medical College (HV 8C2: Ig G2a) in Korea and the other was from the US Army Medical Research Unit/Korea (BE-08: nucleocapsid protein Ig G1). The stain was done by the routine avidin-biotin complex method.

RESULTS

1. Histopathologic findings

On the 3rd day after intracerebral (IC) inoculation there were an increased number of tingible body macrophages beneath the spleen capsule (Fig. 1), which were positive in cytoplasm for HTNV antigen by the indirect immunofluorescent study (Fig. 2a) but were negative by the immunohistochemical stain (Fig. 2b). On the 5th day after IC inoculation, the white pulp began to show a distinct morphology. On the 7th day after IC and after intramuscular (IM) inoculation, the white pulp area was clearly seen and mostly composed of small lymphocytes and some large lymphocytes around the periarteriolar lymphocytic sheath (Fig. 3). On the 9th and 14th days after IM inoculation, there was a pronounced expansion of white pulp (Fig. 4) with a markedly increased number of medium and large lymphocytes in marginal zone, some immunoblasts and plasma cells (Fig. 5). The extramedullary hematopoiesis was gradually decreased from the 5th day until the 9th day in the ICG and the 17th day in the IMG (Fig. 6).

Legends for figures

Fig. 1. On the 3rd day after intracerebral inoculation with Hantaan virus, there is an increased number of macrophages (arrows) below the splenic capsule. (H & E stain, ×400)

Fig. 2a. In the immunofluorescent antibody technique, there are focally scattered positive reactions in the cytoplasm of probable macrophages. (×400)

Fig. 2b. In the immunohistochemical stain, there is no identifiable positive reaction in any cells. (×1000)

Fig. 3. On the 7th day after intracerebral inoculation with Hantaan virus, small lymphocytes and marginal zone around the periarteriolar sheath (arrows) appear to be distinct. (H & E stain. ×200)

Fig. 4. On the 9th and 14th days after intramuscular inoculation of Hantaan virus, marked expansion of marginal zone is surrounding the small lymphocytes. (H & E stain, ×200)

Fig. 5. Within the medium and large lymphocytes zone, scattered immunoblasts (a) and small numbers of plasma cells (b) are admixed. (PAS stain, ×400)

Fig. 6. On the 17th day after intramuscular inoculation of Hantaan virus, the extramedullary hematopoiesis is markedly decreased. (PAS stain, ×400)

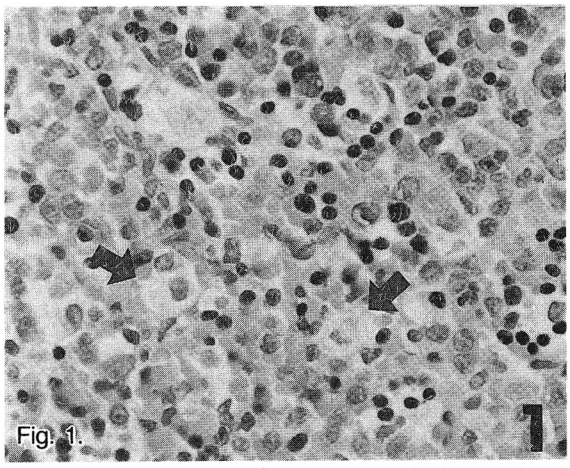

Fig. 1.

Fig. 4.

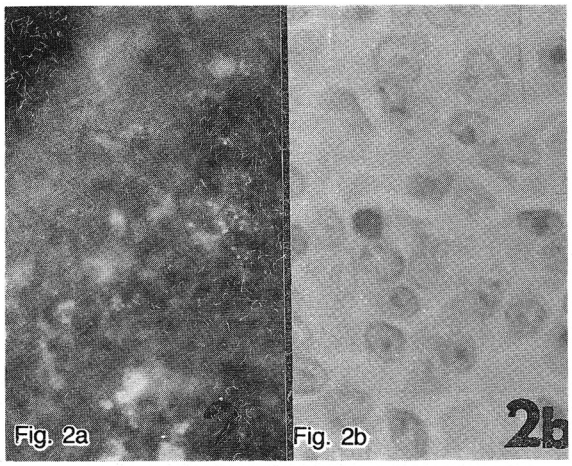

Fig. 2a Fig. 2b

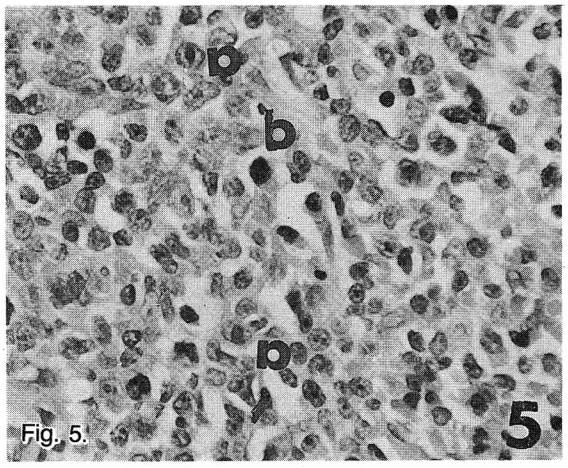

Fig. 5.

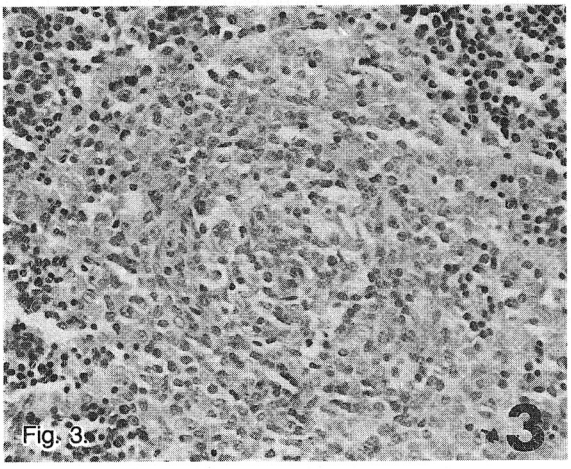

Fig. 3.

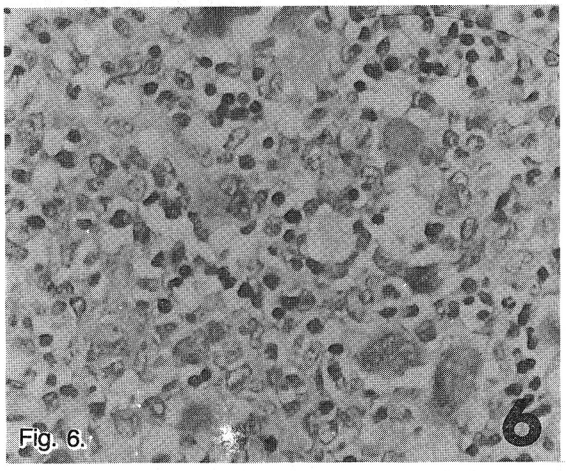

Fig. 6.

Spleen thickness

Both the ICG and IMG showed increased spleen thickness especially from the 5th and the 9th day, respectively. The degree of thickness in the ICG is greater than in the IMG (Fig. 7).

The ratio of white to red pulp

The margin between white and red pulp became distinct after the 5th day. Compared to the control group, the ratios of white to red pulp were slightly increased in the ICG but significantly increased in the IMG (Fig. 8).

The number of rats showing distinct marginal zone after HTNV inoculation

Both groups showed an increased number of rats with distinct marginal zone of the spleen and the ICG had a much greater number of rats at a much earlier time (Fig. 9).

Degree of splenic extramedullary hematopoiesis

The extramedullary hematopoiesis activity is scored from one to three positivities according to the degree of megakaryopoiesis and erythropoiesis. Though the extramedullary hematopoiesis was decreased in both groups, the ICG demonstrated remarkable change at an earlier time than the IMG (Fig. 10).

2. Immunofluorescent findings

In both ICG and IMG, positive cytoplasmic reaction for HTNV antigen was focally seen in the macrophages below the capsular area from the 3rd day after HTNV

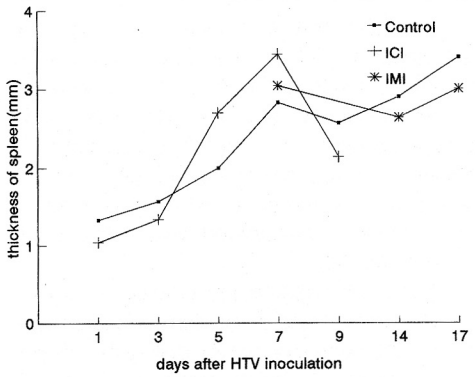

Fig. 7. Spleen thickness of the control and experimental groups in days after HTNV inoculation.

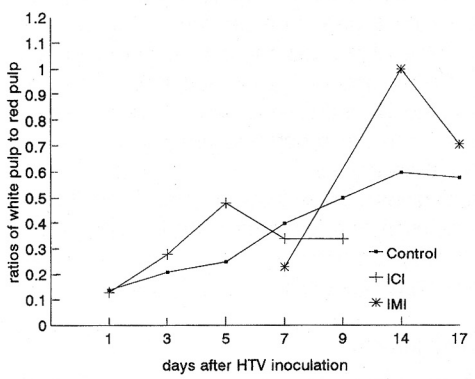

Fig. 8. Ratios of white pulp to red pulp in days after HTNV inoculation

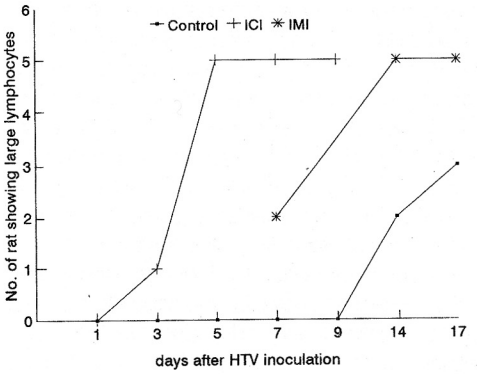

Fig. 9. Number of rat showing distinct marginal zone in days after HTNV inoculation

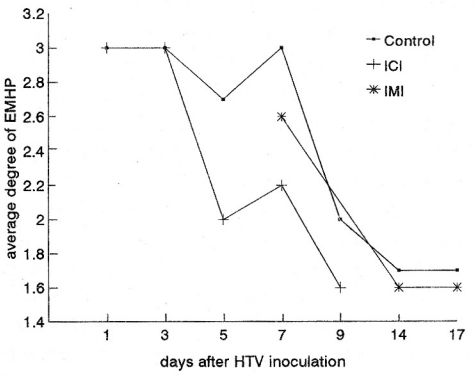

Fig. 10. Degree of splenic EMHP in days after HTNV inoculation

inoculation (Fig. 2a).

3. Immunohistochemical findings

There was no positive reaction in the cytoplasm of any splenic cells by monoclonal antibodies (HTNV 8C2 and BE-08) for HTNV (Fig. 2b).

DISCUSSION

Hemorrhagic fever with renal syndrome is a debilitating disease of human caused by the Hantaan virus, the prototype virus of a newly proposed genus of Hantavirus (Schmaljohn et al, 1983).

Serologic (Gajdusek, 1982) and virologic (Leduc et al, 1984) findings have established the presence of Hantaan-like viruses in rodents virtually worldwide. Studies of HTNV pathogenesis have been limited by the absence of a well defined model for a virus-induced disease state. But, it has recently been learnt that newborn outbred suckling mice are shown to be consistently susceptible to lethal infection with non-mouse adapted HTNV. The clinical courses, mean time to death and fatal outcome are age-dependent (Tsai et al, 1982; Kurata et al, 1983; Kim et al, 1985). While a lethal rodent model is not similar to the human clinical syndrome, its development, characterization and exploitation should nonetheless provide a valuable insight into the mechanisms of this disease.

Our previous examination by light microscopy and immunofluorescence to establish the distribution of viral antigen after infection found HTNV disseminated in various organs, which was evidenced by the presence of viral antigen in several organs although HTNV antigen was detected less consistently and not in every animal in the spleen compared to the brain and the lungs (Cho et al, 1991). The histopathologic changes were much more severe in the IMG and this was interpreted as the result of the late period forming a more mature immune system for HTNV. However, no differences in the pattern of immunofluorecence could be found between rats inoculated IC and IM, except for the delayed appearance of the virus antigen in the IMG.

As was seen in earlier experiments, the sequence of clinical, virologic, serologic (Kim et al, 1985) and histopathologic events (Mckee et al, 1985; Cho et al, 1991) with the immunohistoperoxidase techniques and enzyme-histochemistry (Eikelenboom et al, 1985) suggested a possible immunologic basis for the disease, especially by T lymphocytes. In the present study we used the spleen to evaluate the immunologic state because the spleen is the largest lymphoid organ in rats and white pulp constitutes the immunologically active compartment. Twenty-five suckling rats of less than twenty four hours of age inoculated IC for the early change and fifteen suckling rats inoculated 1M for the late change were consistently susceptible to lethal infection with the seed HTNV.

The first lesion to appear in chronological sequence was appearance of macrophages, especially below the capsule, from the 3rd day after HTNV inoculation. Small lymphocytes appeared with some immunoblasts and plasma cells around the periarteriolar sheath on the 5th day increasing in numbers on the 7th day. After that, marginal (medium and large lymphocytes) zone around the small lymphocytes became remarkably expanded with decreased small lymphocytes and extramedullary hematopoiesis on the 9th and 14 days.

The appearance of macrophages at an early stage probably indicates the virus uptake (Kim et al, 1987), and the increase of small lymphocytes on the 7th day, which is reported to be the peak period of viremia (Lee et al, 1986), can be correlated with the proliferation of T lymphocytes. The remarkable increase of large lymphocytes with immunoblasts and plasma cells on the 9th and 14th days, reported to be the peak period of antibody formation for Hantaan virus (Kim et al, 1988), can be correlated with the marked proliferation of B lymphocytes. The evidence of decreasing extramedullary hematopoiesis on the 9th and 14th days is considered to be the result of expanded white pulp and the ageing effect after birth.

Time to onset of histopathologic changes in spleen thickness, the appearance of medium and large lymphocytes and the degree of extramedullary hematopoiesis were influenced by inoculation route, whereas expansion of the marginal zone was affected by postnatal age. Rats inoculated IC displayed somewhat greater susceptibility and shorter duation of disease, while those inoculated IM showed slightly enhanced resistance and a delay in mean time to death.

In the spleen there was only a small number of detectable HTNV antigens after inoculation, this was demonstrated by more senstive immunofluorescent technique than immunohistochemical stain using monoclonal antibodies.

These findings suggest that the spleen is not the site of the viral reservoir or proliferation and that the spleen seems to play a major role in the immune response following HTNV inoculation in suckling rats.

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