Hepatic Lesions in Cattle Caused by Experimental Infection of *Fusobacterium necrophorum*

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(Received 14 May 1985/Accepted 15 February 1986)

ABSTRACT. Inoculation of *Fusobacterium necrophorum* into the portal venous system of cattle resulted in the formation of microabscesses and disseminated microthrombi in the liver. The microabscesses expanded with multiplication of *F. necrophorum*, resulting in the formation of coagulative necrosis. The areas were encapsulated with granulation tissues. The development of hepatic lesions was partially associated with intravascular coagulation in the liver.—**KEY WORDS:** DIC, *Fusobacterium necrophorum*, liver abscess.

Jpn. J. Vet. Sci. 48(3): 509–515, 1986

Fusobacterium necrophorum, one of the normal bacterial flora in the rumen, has been considered to cause liver abscess by entering the blood stream through the rumenal lesions [1, 7–9, 11]. Liver abscess have been produced experimentally by inoculation of *F. necrophorum* into the portal venous system of cattle and sheep, but not via the jugular vein [6, 17, 18]. These facts suggest that a type of liver abscess in beef cattle is the result of an endogeneous infection. Pathologically, coagulative necrosis in hepatic parenchyma is caused by multiplication of *F. necrophorum*, and the necrotic area turns to abscess in cattle and sheep [6, 21]. Similar coagulative necrosis and abscess in the liver have also been produced in mice, in which the lesions are caused by bacterial toxins as well as bacterial embolism [4, 16]. However, the process of the formation of the coagulative necrosis has not been sufficiently described in detail. The purpose of this experiment was to reappraise the pathological nature of hepatic lesions caused by *F. necrophorum* in cattle. The bacteriological results of a part of this experiment have already been published [18].

MATERIALS AND METHODS

F. necrophorum type A, ATCC 25286, was grown in the modified viande et de levures (VL) broth at 37°C for 18 hours using the roll tube method. It was collected by centrifugation, washed once with the broth and resuspended in fresh broth for the inoculation [16, 18]. The bacterial suspension was inoculated into the ruminal or abomasal vein of 12 cattle by laparotomy [18]. The inoculated doses ranged from 10⁷ to 10¹¹ colony forming units (CFU).

Necropsy was done between 12 hrs and 21 days after the inoculation. Samples taken from various organs were fixed in 10% buffered formalin. Tissue samples from the liver were also fixed in 95% cold ethanol for indirect immunofluorescence [16]. Paraffin sections were stained with hematoxylin and eosin (HE) or Gomori's methenamine silver stain (GMS). Three to five sections of liver were also stained with phosphotungstic acid-hematoxylin (PTAH) and Fraser-Lendrum's fibrin stain (FL). Excluding the abscesses, the maximum number of fibrin thrombi per

Table 1. Hepatic lesions in cattle inoculated with *F. necrophorum*

No.	Age of Cattle	Dose ^{a)}	D.P.I. ^{b)}	Hepatic lesions			Kim's ^{c)} Score
				Abscess	Thrombi	Microthrombi	
1	1 month	10	0.5(D) ^{d)}	Microabscess	0 ^{e)}	1848	4+
2	1 year	11	1 (D)	Microabscess Necrosis	0	336	4+
3	1 year	9	1.5(D)	Necrosis	26	51	4+
4	1 month	9	2 (S)	Necrosis	20	97	4+
5	2 months	9	4 (D) ^{f)}	Necrosis	11	19	4+
6	10 months	8	9 (S)	Abscess	0	3	2+
7	6 years	8	14 (S)	Abscess	0	0	—
8	2 months	9	21 (S)	Abscess	0	1	1+
9	2 months	8	21 (S)	Abscess	0	0	—
10	2 months	7	21 (S)	Abscess	0	0	—
11	1 month	7	2 (S)	No lesions	0	0	—
12	2 months	7	21 (S)	No lesions	0	0	—

a) logCFU/10 ml.

b) Days post inoculation.

c) Index of fibrin thrombi: 1+=1 to 2 thrombi, 2+=3 to 4, 3+=5 to 10, 4+=more than 10 thrombi, respectively.

d) (D) means died and (S) means sacrificed.

e) Arabic numerals mean number of thrombi.

f) Calf No. 5 died of hernia.

PTAH stained section (1 cm²) was counted and scored according to Kim's standard as follows: one or two as 1+; three or four as 2+; four to ten as 3+; more than ten as 4+ [10, 20]

RESULTS

Gross lesions: Hepatic lesions were observed in cattle inoculated with 10⁷ CFU of *F. necrophorum* or more as shown in Table 1. At 12 hrs after the inoculation, white foci of 0.5 to 1 mm in diameter were seen disseminated throughout the liver of No. 1 cattle. Circumscribed white to yellowish foci, 2 to 3 mm in diameter, were frequently found in the livers of Nos. 2 and 3 at 24 to 36 hrs after the inoculation. The circumscribed foci grew to 1 cm in diameter at 2 to 4 days after the inoculation, and they were caseous, irregularly rounded in shape and slightly elevated from the surface of the livers of Nos. 4 and 5. Congestions or hemorrhages, as well as cloudy swelling, were also seen in the livers

of No. 1 to 4.

At 9 days or more after the inoculation, 3 to 10 abscesses, which ranged between 0.6 and 10 cm in diameter, were seen in the livers of Nos. 6 to 10. The abscesses consisted of caseous tissues, pus and granulation tissues.

Petechial hemorrhages were seen in the skeletal muscles, heart, urinary bladder, gall bladder, and lungs of Nos. 1 and 2 cattle died within 24 hrs after the inoculation. Lung abscesses were found in Nos. 8 to 10.

Microscopic lesions: At 12 hrs after the inoculation, microabscesses and microthrombi were seen disseminated in the sinusoids of No. 1 cattle (Table 1, Fig. 1). *F. necrophorum* were pleomorphic in shape (Fig. 2) and often formed tiny bacterial colonies in the microabscesses with GMS. Immunofluorescent staining also showed the tube-like or homogeneous antigens in the microabscesses. Eosinophilic substances, a few degenerated neutrophils and macrophages were observed around *F. necrophorum*. Hepatocytes were often involved in the microabscesses. The

Fig. 1. Microabscess containing the embolus of *F. necrophorum* (left), and microthrombus (right) in the sinusoids of No. 1. Hematoxylin and eosin (HE) staining $\times 800$.

Fig. 2. *F. necrophorum* in the microabscess of No. 1. Gomori's methenamine silver staining $\times 480$.

eosinophilic substance was not stained with PTAH and FL. Microthrombi were located around the microabscesses and were more frequently observed near the portal triads (Fig. 3). These microthrombi were stained with PTAH and FL, and were thread or strand-like in shape. At 1 day after the

Fig. 3. Microthrombi are located in the sinusoids at the peripheral zone of the hepatic lobules of No. 1. Phosphotungstic acid-hematoxylin (PTAH) staining $\times 120$.

Fig. 4. The focus of coagulative necrosis, appearing pale in this photograph, contains nuclear debris in the center in No. 3. PTAH staining $\times 48$.

Fig. 5. Inflammatory cell infiltration around the necrotic focus in No. 5. HE staining $\times 240$.

inoculation, the hepatic tissues around the microabscess showed coagulative necrosis in No. 2, while infiltration of neutrophils and macrophages was minimal around the necrotic foci. Hemorrhages and microthrombi of fibrin were seen in and around the necrotic foci, as well as in the sinusoids (Table 1).

At 1.5 to 4 days after the inoculation, the necrotic foci were enlarged in size and often fused together in Nos. 3 to 5. Substantial nuclear debris, *F. necrophorum* and a homogeneous tissue mass were observed at the center of the necrosis (Fig. 4). The sinusoids in the necrotic foci were filled with filamentous *F. necrophorum*. Tube-like antigens were clear in the peripheral zone of the necrotic foci and were discernible in the center by immunofluorescence. Infiltration of neutrophils and macrophages around the necrotic foci was slight in No. 3 and mild in Nos. 4 and 5 cattle (Fig. 5). Swelling of endothelial

cells and infiltration of neutrophils or macrophages were observed in the walls of arteries and veins of the portal triads in Nos. 4 and 5. In addition, foci of necrobiosis of the hepatocytes showed scattered distribution in Nos. 3 to 5. The hepatocytes were shrunken and unusually eosinophilic, and almost all of their nuclei showed hyperchromatosis. Karyorrhexis or pyknosis appeared scattered. Fibrin thrombi, numerous neutrophils and macrophages were also observed in the sinusoids and small vessels (Fig. 6), while replication of *F. necrophorum* was minimum. The lesion of necrobiosis was located adjacent to the coagulative necrosis with multiplication of *F. necrophorum*, or was focal or zonal with frequent involvement of the central veins but not the portal triads (Fig. 7). Neutrophils and macrophages increased in numbers in the sinusoids. Fibrin thrombi, homogeneous or reticulated in shape, were seen scattered in the veins of the portal triads, as well as the

Fig. 6. Microthrombi and reticulated thrombi in the necrobiotic focus of No. 4. PTAH staining $\times 240$.

Fig. 7. Zonal and focal necrobiosis containing numerous neutrophils and macrophages in No. 4. HE staining $\times 120$.

microthrombi of fibrin in the sinusoids (Table 1).

At 9 days or more after the inoculation, liver abscesses, which contained coagulative necrosis, a large number of *F. necrophorum* and nuclear debris in the hepatic tissues, were encapsulated with fibrinous exudates, plasma cells and fibrous tissues in Nos. 6 to 10. Mild perivascular infiltration of plasma cells and neutrophils was also seen in the portal triads. Fibrin strands were less frequently observed in the sinusoids of Nos. 6 and 8 (Table 1).

In the organs other than the liver, fibrinous exudation with infiltration of neutrophils was observed in the lungs of No. 5, and abscess in Nos. 8 through 10. Congestion with occasional microthrombi of fibrin in the lungs and swelling of macrophages in the red pulp of the spleen were seen in Nos. 1 through 5. Diffuse lymphocytic necrosis in the spleen and lymph nodes was observed in addition to gross lesions of hemorrhages in Nos. 1 and 2.

DISCUSSION

Coagulative necrosis and its encapsulation have been described in liver abscess of cattle [4, 6, 11, 16, 18, 21]. However, the pathologic process leading to coagulative necrosis has not been reported in detail. Recently, microabscess and bacterial emboli in the liver were claimed as initial lesions in an experimental *F. necrophorum* infection in cattle [17]. In the present study, microabscess was seemingly induced by the emboli of *F. necrophorum* in the sinusoid, and the lesion developed to coagulative necrosis by involving adjacent hepatic tissues with multiplication of *F. necrophorum*. The foci of coagulative necrosis were later encapsulated with granulation tissues.

In the early stage of the present infection, microthrombi made of fibrin were observed in the sinusoids around the periportal area as well as fibrin thrombi in the small vessels.

Previous results with mice indicated that intact *F. necrophorum* caused focal coagulative necrosis in association with fibrin thrombosis around the necrotic foci, and that the components of *F. necrophorum* caused disorder in blood coagulation [16]. The endotoxin from *F. necrophorum* elicited both the generalized and local Shwartzman reaction, in which the lesions were characterized by fibrin thrombosis [3, 5]. The present results showed an association of the focal or zonal necrobiosis with minimum bacterial replication. Histopathologically, these lesions resembled those in acute hepatic necrosis produced by endotoxin, in which the mechanism has been proposed as the uni-visceral Shwartzman reaction or focal intravascular coagulation (FIC) [13, 14]. The components of *F. necrophorum* induced similar hepatic necrosis to those produced by so-called uni-visceral Shwartzman reaction in rabbits (unpublished data). A histopathological criterion of disseminated intravascular coagulation (DIC) is the presence of fibrin thrombi [10, 20]. Intravascular coagulation and its sequences with single-organ involvement have been proposed as local DIC or FIC [12, 14]. On the other hand, DIC, with multiple-organ involvement, has been found in various diseases including septicemia, and has been reproduced by endotoxin in experimental animals and cattle [2, 15, 19]. The present results suggest that development of the hepatic microabscesses into the foci of coagulative necrosis along with multiplication of *F. necrophorum* was partially associated with intravascular coagulation in the liver, which was designated as so-called local DIC or FIC.

ACKNOWLEDGEMENTS. We express our thanks to Prof. Y. Fujimoto, the Department of Comparative Pathology, Hokkaido University, for his helpful advices, and Drs. Y. Isayama, K. Hashimoto and S. Terui, and Messrs. M. Wada, M. Ito and Y. Ando of our Institute, for their technical assistances.

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要 約

Fusobacterium necrophorum 実験感染による牛の肝臓病変：中島靖之・上田 久・八木行雄・中村菊保¹⁾・元井霞子¹⁾・竹内正太郎²⁾（家畜衛生試験場東北支場，¹⁾家畜衛生試験場，²⁾家畜衛生試験場北陸支場）——門脈系への *F. necrophorum* の接種によって肝臓に微小膿瘍と播種性の微小血栓が形成された。微小膿瘍は *F. necrophorum* の増殖につれて凝固壊死となり，肉芽組織によって被包されていた。肝病変形成過程の初期に血管内凝固の関与がみられた。