In the control liver, NLDC-145-positive hepatic DC were located mainly in the portal area and were rarely seen in the parenchyma (Fig. 3a). The density of these hepatic DC gradually decreased with the duration of griseofulvin feeding (Fig. 4). After 6 weeks, the DC disappeared in most of the portal area (Fig. 3b), and the density of the hepatic DC was less than 10% of that in the controls (Fig. 4). However, the index of the number of DC in the whole liver showed a different course of changes, increasing slightly until 4 weeks, but falling sharply at 6 weeks (Fig. 5).

On the other hand, F4/80-positive cells in the control liver were scattered in the hepatic parenchyma and the portal area (Fig. 6a). Accumulation of macrophages in the portal area was obvious at 3 weeks of griseofulvin feeding, but the increase in the density of Kupffer cells in the sinusoidal area was relatively gradual (Fig. 7). At 8 weeks on the griseofulvin diet, Kupffer cells were sometimes hypertrophic, and clusters of Kupffer cells often contained brown pigment deposits (Fig. 6b). In this stage, the density of Kupffer cells was significantly increased (Fig. 7).

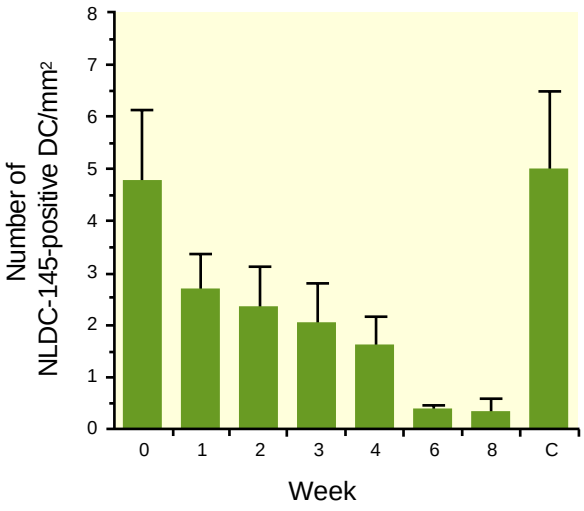

Fig. 4. Changes in the density of NLDC-145-positive DC in the liver after griseofulvin feeding. The density is significantly decreased after 1 week. Data are expressed as mean ± SD. Bars represent SD. C, 13-week-old control mice.

In the spleen and lung, no significant differences were noted in the distribution of NLDC-145-positive DC between experimental and control mice (Figs. 8 and 9). These DC were located mainly in the periarterial lymphoid sheath in the spleen, and studded the lung parenchyma.

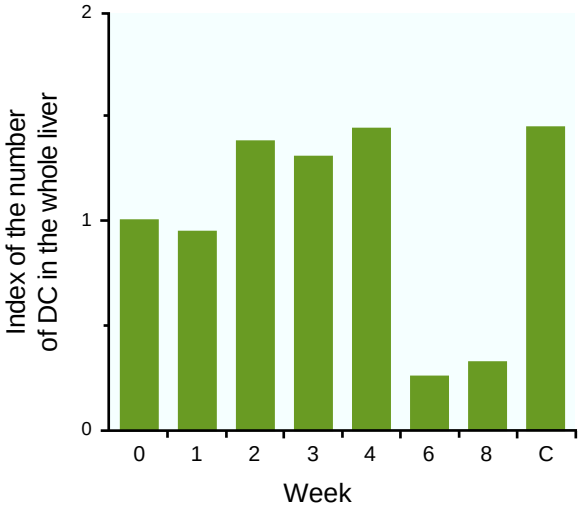

Fig. 5. Changes in the index of the number of DC in the whole liver after griseofulvin feeding. The mean value of the products [cell density (/mm²) by liver weight (g)] was calculated in each stage, and the ratio of the value to that of the 5-week-old controls was evaluated. After 6 weeks, the index falls sharply. C, 13-week-old control mice.

Discussion

This study showed a decrease in density of NLDC-145-positive hepatic DC and an increase in that of F4/80-positive Kupffer cells in griseofulvin-induced protoporphyric liver injury.

The decrease in density of hepatic DC indicated a reduction of hepatic DC per unit volume of the liver. However, this change does not imply a decrease in number in the whole liver. Hepatomegaly, an increase in the volume of the liver, might be responsible for the decrease in density of hepatic DC. Therefore, we calculated the index of the number of DC in the whole liver. Hepatic DC number in the whole liver appeared to increase slightly until 4 weeks of griseofulvin treatment and fell sharply after 6 weeks. These results suggested that the decrease in density of hepatic DC was due to hepatomegaly until 4 weeks of treatment, and was substantial after 6 weeks.

In contrast to the decrease in number of hepatic DC, there were no definite changes in the distribution of NLDC-145-positive DC in the spleen or lung. This implied that the decrease in number of NLDC-145-positive DC was specific in the liver. To evaluate these observations, we considered the results of previous reports showing the behavior of circulating DC and turnover time of tissue DC. Intravenously transferred DC migrate mainly to the liver and spleen, and partly to the lungs and other organs (Fossum, 1988; Kupiec-Weglinski et al., 1988; Suda et al., 1996). A physiological study further showed that the turnover time of mouse splenic DC is 8–11 days (Steinman et al., 1974). If the decrease of hepatic DC was due to a reduction in number of circulating DC, splenic DC should have decreased in number in the later stage. However, splenic DC showed no definite changes after 8 weeks of griseofulvin feeding, suggesting that the number of circulating DC does not decrease in protoporphyric mice at least until about 2 months. Accordingly, it is reasonable to speculate that some changes that occurred in the injured liver caused the specific decrease in hepatic DC number in protoporphyric mice.

The liver is an important organ for blood DC to migrate to the lymph. Blood DC attach to the vessel wall in the sinusoidal area, pass through the space of Disse to the connective tissue stroma of either the portal or hepatic vein area, and then enter the initial lymphatic ducts accumulating in the regional hepatic lymph nodes via the afferent lymph (Kudo et al., 1997). It

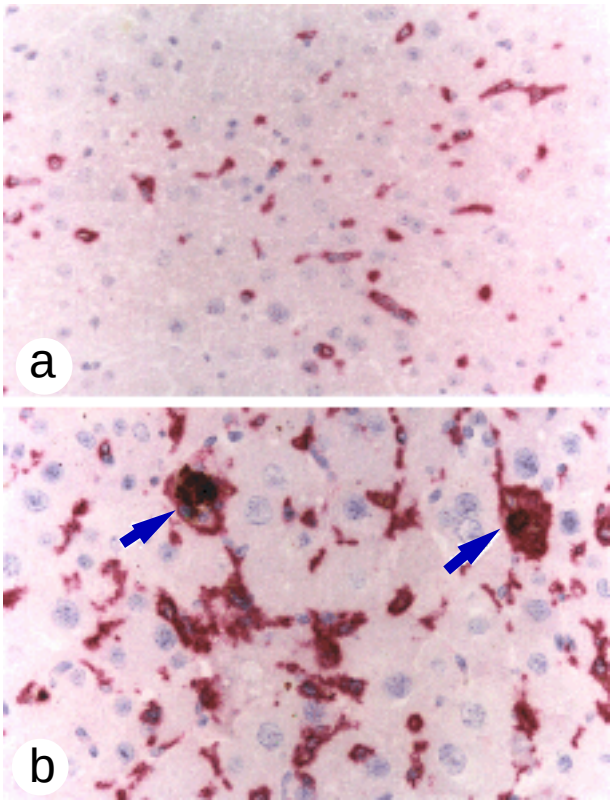

Fig. 6. Immunostaining of the liver with monoclonal antibody F4/80 for Kupffer cells (red). **a**: A 5-week-old control mouse. F4/80-positive Kupffer cells are distributed in the sinusoid. × 250. **b**: A mouse treated with griseofulvin for 8 weeks. F4/80-positive Kupffer cells are hypertrophic and markedly increased in number. Note clusters of Kupffer cells containing brown pigment deposits (arrows). × 250.

is probably important for migration that the normal hepatic structure and function are maintained. Alteration of liver structure after 4 weeks of griseofulvin feeding might interrupt the migration pathway and subsequently cause the decrease of DC in the whole liver. After 4 weeks of griseofulvin feeding, marked ductular reaction occurred in the injured liver (Shapiro and Wessely, 1984). This reaction is a characteristic change observed in the cholestasis of bile duct obstruction (Slott et al., 1990; Desmet, 1995; Roskams and Desmet, 1998). Recently, Tanimoto et al. (1999) reported that DC numbers were extremely low in the livers of patients with large bile duct obstruction. This implies that ductular reaction might cause a decrease in number of DC in the whole liver. However, ductular reaction has also been shown to occur in other liver diseases in which hepatic DC increase in number (Popper, 1986; Roskams and Desmet, 1998). Therefore, we could not conclude that the ductular reaction we found led to a decrease of hepatic DC in protoporphyric mice.

DC have been suggested to infiltrate the liver and then play a pivotal role in the development of chronic inflammatory liver diseases such as primary biliary cirrhosis, chronic hepatitis type B and type C, and autoimmune hepatitis (van den Oord et al., 1990; Kaji et al., 1997; Shinomiya et al., 1998; Tanimoto et al., 1999). In contrast, hepatic DC seem to have no significant relation to the progression of griseofulvin-induced protoporphyric liver injury as these cells decreased in number in this type of liver injury.

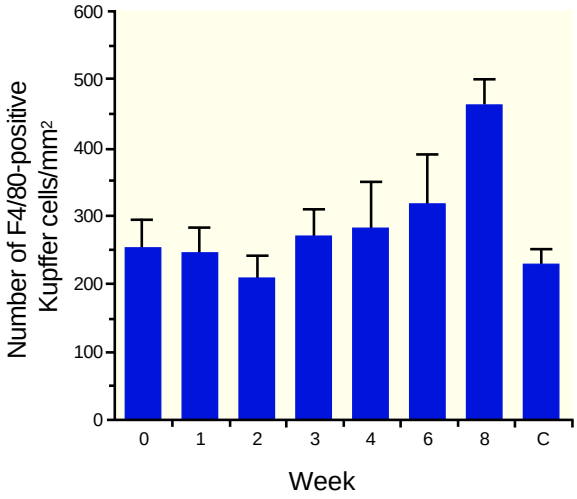

Fig. 7. Changes in the density of F4/80-positive Kupffer cells after griseofulvin feeding. The density of these cells shows a significant increase at 8 weeks. Data are expressed as mean ± SD. Bars represent SD. C, 13-week-old control mice.

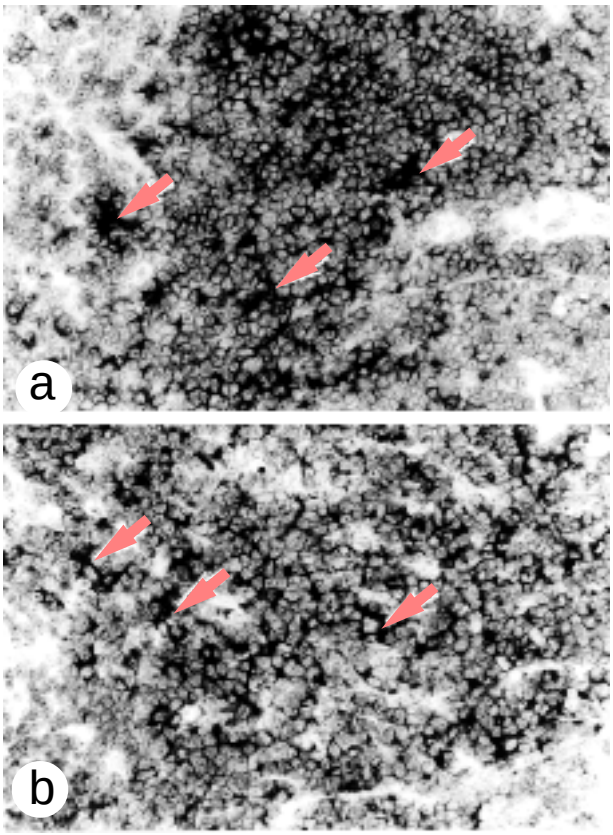

Fig. 8. NLDC-145-positive DC in the spleen of a 5-week-old control mouse (**a**) and a mouse treated with griseofulvin for 8 weeks (**b**). There are no significant differences in the distribution of splenic DC between the control and experimental animals. The splenic DC are distributed in the periarterial lymphoid sheath (arrows). × 280.

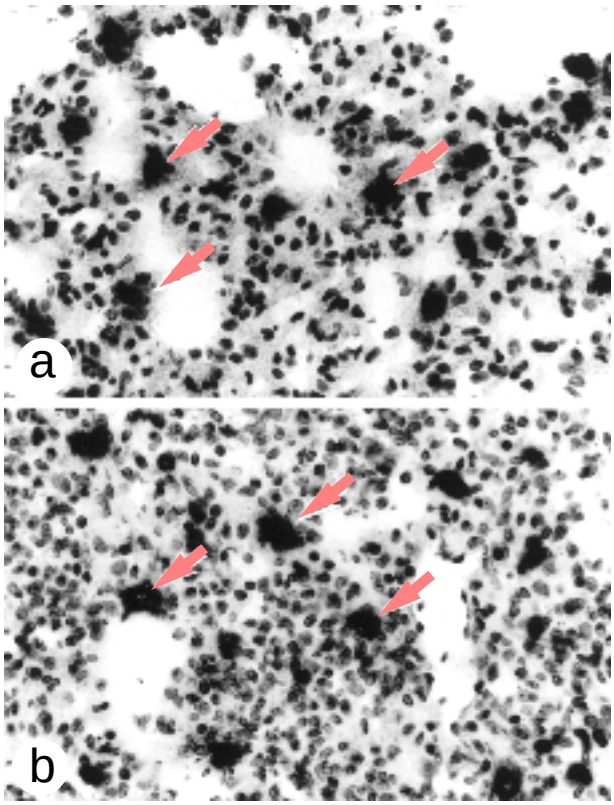

Fig. 9. NLDC-145-positive DC in the lung of a 5-week-old control mouse (**a**) and a mouse treated with griseofulvin for 8 weeks (**b**). In these sections, DC show a similar distribution in the parenchyma (arrows). × 280.

These observations raise the question of whether the decrease in hepatic DC number affects the immune system in protoporphyric mice. Previous studies showed that griseofulvin-treated mice were susceptible to bacterial infection (Weston-Hurst and Paget, 1963; Mitchell et al., 1973), and suggested a selective depressing effect of griseofulvin on the cellular immune response in mice (Mitchell et al., 1973). Recently, Matsuno et al. (1996) suggested that DC phagocytosed intravenously injected particles in the blood marginating pool and translocated from the sinusoidal area to the hepatic lymph, and these cells might initiate immune responses against pathogenic organisms such as bacteria and fungi. The susceptibility to bacterial infection and the depression of the cellular immune response in griseofulvin-treated mice might be due to the decrease in number of hepatic DC.

In protoporphyric mice, Kupffer cells are activated to phagocytose protoporphyrin crystals (Gschnait et al., 1975). However, quantitative analysis of Kupffer cells has not been performed. There are no precise markers for mouse Kupffer cells. In this study, we used a monoclonal antibody, F4/80, which recognizes macrophages including Kupffer cells in mice. To estimate the density of Kupffer cells in the liver, we counted the F4/80-positive cells only in the sinusoidal area and excluded macrophages in the portal area. Our results showed that the density of Kupffer cells gradually increased in the injured liver. Considering the effect of hepatomegaly on the density of Kupffer cells, the number of Kupffer cells in the whole liver probably increased more significantly than their density revealed. However, we could not calculate the index of the number of Kupffer cells in the whole liver from the product of their density by liver weight because they were located only in the sinusoidal area.

Recently, it was reported that Kupffer cells are involved in the migration of hepatic DC; i.e. Kupffer cells were shown to be capable of selectively trapping DC from the blood (Suda et al., 1996; Kudo et al., 1997). In the present study, since densities of DC and Kupffer cells showed opposite changes in the injured liver, the decrease of hepatic DC seemed to have no relation to Kupffer cells. However, we could not eliminate the possibility that Kupffer cells failed to trap circulating DC in the protoporphyric liver. Further studies are needed to determine whether Kupffer cells in protoporphyric mice can trap DC from the blood.

In conclusion, we demonstrated a decrease in number of hepatic DC and an increase in number of Kupffer cells in protoporphyric liver injury using an experimental mouse model and immunohistochemical methods. It seems that

the decrease in density of hepatic DC is due to hepatomegaly until 4 weeks of griseofulvin treatment, and is substantial after 6 weeks. Alteration of the liver structure might cause this substantial decrease in number of hepatic DC. Although hepatic DC seemed to have no relation to the progression of the griseofulvin-induced protoporphyric liver injury, their decrease might affect the cellular immune response in protoporphyria.

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