The Protective Effect of Cyclosporine A on Anti-Fas Antibody-Induced Hepatitis in Mice

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ABSTRACT—The effect of cyclosporine A (CsA) on anti-Fas antibody-induced hepatitis was studied. The administration of anti-Fas antibody (250 μ g/kg) to mice elevated plasma alanine aminotransferase (ALT) activity at 3 hr. This anti-Fas antibody-induced elevation of ALT was inhibited by treatment with CsA (10, 30 and 100 mg/kg) in a dose-dependent manner. Anti-Fas antibody administration elevated CPP32-like protease activity at 3 hr in mouse liver, and this elevation of CPP32-like activity was inhibited by treatment with CsA. The present results show that CsA treatment inhibits the anti-Fas antibody-induced apoptotic process of hepatitis, at least in part, by affecting a reaction upstream of CPP32-like protease activation.

Keywords: Fas, Cyclosporine A, Liver

Liver transplantation is a powerful therapeutic approach for prolonging the life of a patient who has severe hepatic failure. In liver transplantation, the prevention of allogenic rejection using adequate immunosuppressants is critical. The rejection of allografts depends on the activation of alloreactive T-cells (1), and cyclosporine A (CsA) has been extensively used to inhibit allograft rejection in liver transplantation (2). Alloreactive T-cell activation is mediated through CD28, which is a receptor of co-stimulator factors for allogenic T-cell activation (2). Recently, in addition to the CD28-dependent pathway of allogenic rejection, CD28-independent allogenic rejection was reported (3). Furthermore, the Fas/Fas ligand system reportedly contributes to the CD28-independent allogenic rejection (3). Since CsA therapy is extremely effective in liver transplantation, it is important to determine whether or not CsA treatment inhibits Fas/Fas ligand-mediated hepatocyte rejection in liver transplantation. In order to address this question, we examined the effect of CsA on hepatocyte injury induced by anti-Fas antibodies.

Female BALB/c mice obtained from Charles River Japan, Inc. (Atsugi) were used at 7–10 weeks of age. The animals were kept in an air-conditioned room and given water ad libitum. Anti-Fas antibody Jo2 was purchased from Pharmingen (San Diego, CA, USA). Anti-Fas antibody (250 μ g/kg) was administered to the mice via a tail vein (in a volume of 100 μ l). CsA was obtained from Wako Pure Chemical Industries (Osaka) and intra-

peritoneally injected. The measurement of plasma alanine aminotransferase (ALT) and CPP32-like activity and the

Fig. 1. Effect of CsA treatment on anti-Fas antibody-induced elevation of plasma ALT. Anti-Fas antibody was injected and plasma was obtained from each mouse at 3 hr after the administration. Pretreatment with CsA was performed 1 hr before anti-Fas antibody treatment. Data represented the mean $\pm$ S.E.M. of ALT in plasma obtained in each treatment. Normal: Non-treated ($n=2$). Fas cont: [Anti-Fas antibody (250 μ g/kg, i.v.)]-treated ($n=4$). Fas + CsA: [Anti-Fas antibody (250 μ g/kg, i.v.) + CsA (10, 30 and 100 mg/kg, i.p.)]-treated ($n=4$). * $P<0.01$ vs anti-Fas antibody alone.

histopathological procedures were previously described (4, 5). Polymerase chain reaction (PCR) analysis was performed as described previously (6). Gene-specific primers for interleukin-2 (IL-2) (6) and Fas antigen (4) were previously reported. cDNA was amplified by 30 cycles of denaturation at 94°C for 45 sec, annealing at

55°C for 45 sec, and extension at 72°C for 1.5 min. The results were analyzed by means of the F test, followed by the *t*-test with Welch's correction.

The effect of CsA on anti-Fas antibody-induced hepatitis was examined. The treatment of mice with anti-Fas antibody elevated plasma ALT (Fig. 1). The pretreatment

Fig. 2. Light microscopic photographs of the liver. The liver was removed at 3 hr after anti-Fas antibody injection. CsA pretreatment was performed at 1 hr before anti-Fas antibody administration (H-E staining, $\times 227$). Top: [Anti-Fas antibody (250 $\mu\text{g}/\text{kg}$, i.v.)]-treated. An apoptotic hepatocyte (A) and apoptotic body (B) are indicated by the arrow. Bottom: [Anti-Fas antibody (250 $\mu\text{g}/\text{kg}$, i.v.) + CsA (100 mg/kg, i.p.)]-treated.

of mice with CsA (10, 30 and 100 mg/kg, i.p.) inhibited the anti-Fas antibody-induced elevation of plasma ALT in a dose-dependent manner (Fig. 1). A histopathological study showed anti-Fas antibody-induced apoptotic hepatocytes, which were localized from the periportal to the middle zone. These cells showed a typical apoptotic image such as nuclear condensation, an eosinophilic compact cytoplasm and apoptotic bodies (Fig. 2, top). In contrast, in CsA-treated mice, comparatively intact hepatocytes were observed in the perivenous area, and only some apoptotic cells could be seen in the periportal area (Fig. 2, bottom). CPP32-like protease is involved in the apoptosis induced by various stimuli (7). Elevation of CPP32-like activity plays an essential role in the development of anti-Fas antibody-induced hepatitis (8). The effect of CsA treatment on anti-Fas antibody-induced elevation of CPP32-like activity was studied. Treatment with anti-Fas antibody elevated CPP32-like activity in the mouse liver at 3 hr (Fig. 3A). The pretreatment of mice with CsA (100 mg/kg, i.p.) inhibited the anti-Fas antibody-induced elevation of CPP32-like activity. This in-

dicates that CsA inhibited anti-Fas antibody-induced hepatitis, at least in part, by affecting a reaction upstream of CPP32-like protease activation in the apoptotic process. Since CsA is an immunosuppressant that inhibits T cell activation (9), it is possible that CsA inhibited anti-Fas antibody-induced hepatitis through inhibition of T-cell activation. In the anti-Fas antibody-treated samples, the IL-2 probe detected PCR products. However, judging from the size, real IL-2 mRNA (320 bp) was not amplified (Fig. 3B, a). Thus, T-cell activation may not be involved in the development of anti-Fas antibody-induced hepatitis, and CsA inhibited anti-Fas antibody-induced hepatitis through a mechanism other than the inhibition of T-cell activation. Furthermore, the effect of CsA on Fas antigen mRNA expression was examined. CsA treatment, however, did not inhibit Fas antigen mRNA expression (Fig. 3B, b). Thus, CsA did not inhibit anti-Fas antibody-induced hepatitis by inhibiting Fas antigen expression. CsA reportedly exerts its immunosuppressive effect by inhibiting calcineurin (10). Thus, although activation of the immune system does not seem to be involved in the

Fig. 3. Effects of CsA on CPP32-like activity and cytokine mRNA expression. **A:** Effect of CsA treatment on anti-Fas antibody-induced elevation of CPP32-like activity. Anti-Fas antibody was injected; 3 hr later, the liver was removed and protein was extracted for measurement of CPP32-like activity. CsA pretreatment was performed at 1 hr before anti-Fas antibody administration. CPP32-like activity was measured with the fluorescent substrate, Ac-DEVD-MCA, and liver cytosol extracts. CPP32-like activity was represented as U/50 μ g protein. One unit corresponds to the activity that cleaves 1 pmol of the respective fluorescent substrate at 25°C in 30 min. Normal: Non-treated (n=2). Fas cont: [Anti-Fas antibody (250 μ g/kg, i.v.)]-treated (n=3). Fas + CsA: [Anti-Fas antibody (250 μ g/kg, i.v.) + CsA (100 mg/kg, i.p.)]-treated (n=3). * $P < 0.01$ vs liver cytosol extracts from mice treated with anti-Fas antibody alone. **B:** a: Effect of anti-Fas antibody on IL-2 mRNA. Anti-Fas antibody was injected, and the liver was removed for PCR analysis. The content of the gel lanes from the left are as follows: 1st: 100-bp DNA size marker. 2nd, 3rd, 4th and 5th are liver samples removed at 0, 1, 2 and 3 hr after anti-Fas antibody (250 μ g/kg, i.v.) administration, respectively. b: Effect of CsA on Fas antigen mRNA expression. CsA (50 or 100 mg/kg, i.p.) was administered, and then at 1 or 2 hr, the liver was removed for PCR analysis.

anti-Fas antibody-induced hepatitis in the present study, it is possible that the inhibition of calcineurin in the liver might be involved in the CsA-mediated inhibition of anti-Fas antibody-induced hepatitis.

The present results showed that CsA treatment inhibited anti-Fas antibody-induced hepatitis by affecting, at least in part, a reaction upstream of CPP32-like protease activation. In liver transplantation, in addition to its inhibition of T-cell mediated allogeneic rejection, CsA may also contribute by inhibiting Fas/Fas ligand-mediated allogeneic hepatocyte rejection. These effects of CsA may explain its usefulness in liver transplantation.

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