**In vivo** analysis of Fas antigen-mediated apoptosis: effects of agonistic anti-mouse Fas mAb on thymus, spleen and liver

Yoshiko Nishimura¹, Yoko Hirabayashi², Yumi Matsuzaki³, Philippe Musette³,⁷, Ai Ishii⁴, Hiromitsu Nakauchi³, Tohru Inoue²,⁶ and Shin Yonehara⁵

¹The Pharmaceutical Basic Research Laboratories, JT Inc., Yokohama 236, Japan
²Yokohama City University, School of Medicine, Yokohama 236, Japan
³Department of Immunology, University of Tsukuba, Tsukuba 305, Japan
⁴The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan
⁵Institute for Virus Research, Kyoto University, Kyoto 606-01, Japan
⁶Present address: National Institute of Health Science, Tokyo 158, Japan
⁷Present address: Unite de Biologie Moleculaire du Gene, Institut Pasteur, 75015 Paris, France

**Keywords:** apoptosis, Fas ligand, hepatitis, lpr, T cells, thymic atrophy

**Abstract**

Fas antigen (Fas/CD95) is a cell surface receptor protein that mediates apoptosis-inducing signals. To analyze the function of Fas in vivo, we examined the effects of agonistic anti-Fas antibodies in mice. The i.p. administration of the hamster anti-mouse Fas mAb, RK-8, which induced apoptosis both in vivo and in vitro, did not kill adult mice, whereas those given the another hamster anti-mouse Fas mAb, Jo2, rapidly died of fulminant hepatitis with hemorrhage. Histological analyses of mice given RK-8 indicated severe damage of the thymus, and moderate damage of the spleen and liver. Most of the thymocytes and some hepatocytes underwent apoptosis within 1 day of administration. Flow cytometry revealed that CD4⁺ T cells were more sensitive to Fas-mediated apoptosis than CD8⁺ T cells. At day 7 after administration, the thymus was atrophied. These in vivo effects of RK-8 were transient; the thymus was regenerated, and the liver and spleen were apparently normal 1 month after injection. The administration of RK-8 into newborn mice caused severe damage of the liver and thymus. Most of the hepatocytes died and jaundice was induced. The newborn mice died within 1 week. Most hepatocytes of newborn mice may be more sensitive to apoptosis-inducing signals through Fas than those of adult mice. These results indicated that functional Fas, which introduces the death signal in vivo, is expressed on thymocytes, CD4⁺ splenocytes, and some adult and most newborn mouse hepatocytes.

**Introduction**

Apoptosis, or programmed cell death, plays an important role in many biological processes, including embryogenesis, development of the immune system, elimination of virus-infected cells and the maintenance of tissue homeostasis (1–3). Fas antigen/Apo-1/CD95 (Fas) is a type I cell surface protein that belongs to the nerve growth factor/tumor necrosis factor receptor super family and it can directly transduce an apoptotic death signal into cells by stimulation with agonistic anti-Fas mAb or Fas ligand (FasL) (4–8). Fas mRNA is expressed in many organs including thymus, spleen, liver, heart, lung and ovary of the mouse (8). The expression of Fas protein was analyzed in detail on T and B lineage cells including thymocytes, and peripheral T and B cells (9–12). FasL is a type II membrane protein belonging to the tumor necrosis factor family (7) and the expression of FasL is restricted to activated mature T lymphocytes, except in the testis. Genetic and biochemical analyses of Fas and FasL have revealed that mouse lymphoproliferation (lp) and generalized lymphoproliferative disease (gld) mutations are loss of function mutations of Fas and FasL respectively (13,14). Mice
In vivo effects of anti-Fas mAb

Fig. 1. Cell-killing activity of anti-mFas antibodies, RK-8 and Jo2, in vitro. (a) L5178Y/mFas cells (1 x 10^5 cells/well) were cultured with various doses of anti-mFas mAb (filled squares, RK-8; unfilled circles, Jo2) for 20 h. Mouse thymocytes (b) and hepatocytes (c) were incubated for 20 h with various doses of RK-8 (unfilled circles) or Jo2 (filled squares) anti-Fas antibody in combination with 10 µg/ml cycloheximide. Cell viability was determined by the MTT assay.

Methods

Antibodies

The hamster hybridoma RK-8 that produces anti-mouse Fas mAb was cultured in ASF-104 medium (Ajinomoto, Tokyo, Japan) and the mAb was purified from the culture supernatant without FCS by affinity chromatography on Protein A-Sepharose (Pharmacia, Uppsala, Sweden). Purified anti-mouse Fas antibody (Jo2), control hamster IgG (UC8-4B3), phycoerythrin-conjugated rat anti-L3T4 (CD4) mAb and FITC-conjugated rat anti-Ly2 (CD8) mAb were purchased from PharMingen (San Diego, CA).

Mice

BALB/c, MRL lpr/lpr and MRL +/- mice were purchased from Shizuoka Laboratory Animal Corp. (Hamamatsu, Japan).

Flow cytofluorometry

Single-cell suspensions (1 x 10^6 cells/sample) were incubated for 30 min. on ice in 50 µl of staining buffer (PBS containing 5% FCS and 0.04% NaN_3) containing 20 µg/ml biotinylated anti-mFas mAb, FITC-conjugated anti-CD8 mAb and phycoerythrin (PE)-conjugated anti-CD4 mAb. Two-color cytofluorometry was performed using an EPICS Elite (Coulter Electronics, Hialeah, FL) or a FACSsort (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Cell-killing assay

Cells (1 x 10^5/200 µl) in 96-well microtiter plates were cultured in growth medium containing anti-Fas for 20 h. The cell viability was analyzed by the MTT assay in triplicate and the SD was <5%. The viability was also analyzed by the Trypan blue dye exclusion assay and the results were essentially the same as those of the MTT assay.

Administration of anti-Fas mAb

Purified anti-Fas antibody (5 mg/kg mouse) was i.p. injected into adult mice. The same amounts of control hamster IgG were administrated into control mice. When the mice were <2 weeks old, antibodies (5 mg/kg mouse) were s.c. injected. At various times after injection, peripheral blood was collected and then the animals were sacrificed. The thymuses, spleens, livers, kidneys, intestines, hearts and pancreases were removed and analyzed by histological means. The thymocytes and splenocytes were also analyzed by cytofluorometry.

Histological analyses

The thymus, spleen and liver were fixed in 15% formalin, then sections were stained with hematoxylin & eosin. Liver sections were also stained with azan to analyze the fiber structure.

Isolation of mouse hepatocytes

Hepatocytes were isolated by in situ collagenase perfusion as described (16). In brief, after perfusion with 0.05% collagenase...
Table 1. In vivo effect of anti-Fas mAb administered to newborn and adult mice

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Age</th>
<th>RK-8 a Alive/total (%)</th>
<th>Jo2 a Alive/total (%)</th>
<th>Control Ig a Alive/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>1 day</td>
<td>0/23 c (0)</td>
<td>0/12 d (0)</td>
<td>12/13 (92)</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>0/4 c (0)</td>
<td>4/4 (100)</td>
<td>4/4 (100)</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>9/10 (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>7/8 (88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>5/5 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>5/5 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRL lpr/lpr</td>
<td>1 day</td>
<td>5/5 (100)</td>
<td>0/8 e (0)</td>
<td>5/5 (100)</td>
</tr>
</tbody>
</table>

a Each mAb (5 mg/kg mouse for RK-8, Jo2 and control Ig) was s.c. and i.p. injected into mice younger and older than 2 weeks respectively.
b At day 7 after injection of antibodies, the number of live mice in each group was counted.
c Mice <2 weeks old that received RK-8 died between days 4 and 6 after injection.
d Newborn mice receiving Jo2 died between 18 and 24 h after injection.
e Adult mice receiving Jo2 died within 5 h after injection.

Results

Administration of anti-Fas mAb into mice

Our anti-mouse Fas mAb RK-8, which shows cytolytic activity against cells expressing mFas, specifically recognizes mFas (9). Mouse thymocytes and L5178Y/mFas cells, a mouse T cell line transfected with mouse Fas cDNA, were killed by RK-8 in a dose-dependent manner (Fig. 1a and b). RK-8 was more cytolytic against mouse thymocytes and L5178Y/mFas cells in vitro than the anti-mFas antibody, Jo2. Figure 1(b) shows the cell-killing activity of anti-Fas mAb for murine thymocytes in the presence of cycloheximide. Murine thymocytes, which were highly positive for Fas, were similarly killed by RK-8 in the absence of cycloheximide, although cycloheximide was necessary for the full apoptosis-inducing activity of Jo2 against murine thymocytes (data not shown).

We analyzed the in vivo effects of i.p. administered RK-8. When 5 mg/kg RK-8 was injected into adult and newborn mice, the results were essentially the same as those of 50 mg/kg RK-8, indicating that 5 mg/kg RK-8 showed maximum in vivo effects. Then, we compared the in vivo effect of RK-8 with Jo2 (Table 1). RK-8 (5 mg/kg) had no lethal effects on BALB/c mice >2 weeks old, whereas Jo2 rapidly killed the mice as reported by Ogasawara et al. (15). All the mice given RK-8 survived >1 year. On the contrary, mice 1 day to 1 week old that received 5 mg/kg of RK-8 died between 4 and 6
Fig. 3. Effect of RK-8 administration on the thymus. RK-8 antibody (5 mg/kg mouse) was i.p. injected into 6-week-old adult mice (a–e). The animals were sacrificed before injection (a), and at day 1 (b and c), day 7 (d) and 1 month (e) after injection. RK-8 antibody or control IgG (5 mg/kg mouse) was s.c. injected into newborn mice (f and g, respectively) and the animals were sacrificed 4 days later. Thymus sections were stained with hematoxylin & eosin. Magnification: ×20 (a and e), ×33 (b, d and f), ×50 (g) and ×100 (c).
Effects of RK-8 on the thymus indicated that the RK-8-induced damage of the liver of adult mice was completely different from that of Jo2, which caused death within 5 h. The administration of 1 mg/kg mouse of RK-8, however, had no lethal effect on newborn mice (data not shown). Newborn mice receiving 5 mg/kg mouse of RK-8 developed jaundice from day 2 after injection and at day 3 they were about half the size of control mice (Fig. 2). Newborn mice of MRL pr/pr strain, which expressed very low levels of Fas, were not killed by the administration of RK-8 (Table 1), although wild-type MRL +/+ newborn mice were killed (data not shown).

Another of our anti-mFas mAb RMF-2 (rat IgG1) with associated apoptosis-inducing activity in vitro showed essentially the same in vivo effects as those of RK-8, although our other hamster anti-mFas mAb P4-4, C6-1 and SK-8, which recognize mouse Fas but do not exhibit apoptosis-inducing activity in vitro (9), showed no significant in vivo effects on adult mice (data not shown). When RK-8 was i.v. injected into mice, essentially the same results as i.p. injection were obtained (data not shown).

Effects of RK-8 on spleen

As shown in Fig. 5(a), the T cell area in white pulp narrowed in the spleen of adult mice 1 day after an injection of RK-8. The absolute number of T cells in the spleen decreased to about one-third that of normal mice (data not shown). The number of CD4+ T cells decreased more dramatically than that of CD8+ T cells (Fig. 5c), indicating that the former were more sensitive to the apoptosis-inducing activity of RK-8 in vivo than the latter. We reported that both CD4+ and CD8+ splenic T cells are insensitive to RK-8 in vitro (9). The apoptosis-inducing activity of RK-8 to spleen T cells in vivo seems to be more powerful than that in vitro. The spleen after 1 month injection of RK-8 also recovered completely (data not shown). RK-8 did not significantly affect the spleen of newborn mice, where there are few mature T cells (data not shown).

Effects of RK-8 on liver

Liver sections of adult mice after 1 day injection of RK-8 showed focal damage to hepatocytes and local hepatitis without hemorrhage (Fig. 6b). Figure 6(e) shows the characteristics of apoptosis (fragmented nuclei and acidophilic bodies) on hepatocytes at day 1. There was no infiltration of lymphocytes and granulocytes into the liver, and no fulminant hepatitis with hemorrhage, which is induced by anti-mFas mAb Jo2 (15). Seven days after the injection of RK-8, fiber structures were evident in the liver, which might result from death of hepatocytes (Fig. 6c and f). One month after the injection of RK-8, the liver recovered completely (Fig. 6d) like the thymus and spleen. Biochemical analysis of mouse serum showed that the amount of glutamic oxaloacetic transaminase (GOT) and glutamate pyruvic transaminase (GPT) in the serum dramatically increased 24 h after the injection of antibody, then began to decrease to normal levels (Fig. 7). GOT and GPT in the serum of mice injected with Jo2 rapidly increased. When the levels of GOT and GPT in the serum were higher in mice injected with RK-8 than with Jo2 (Fig. 7). These results indicated that the RK-8-induced damage of the liver of adult mice was significant, but partial and transient. On the contrary, almost all hepatocytes in the liver of newborn mice receiving RK-8 were significantly damaged (Fig. 6g) and spots of bilirubin were evident (Fig. 6h), indicating severe damage to the hepatocytes. The death of newborn mice injected with RK-8 must be associated with the severe damage to the liver. The hepatocytes of newborn mice were more sensitive to the cell death-inducing signals through Fas than those of adult mice in vivo, although fulminant hepatitis with hemorrhage did not develop in adult and newborn mice injected with RK-8.

Effects of RK-8 on thymus

Histological examinations after the administration of RK-8 revealed severe damage in the thymus of adults (Fig. 3). At day 1 after the injection of RK-8, most thymocytes were dead and there were many apoptotic bodies (Fig. 3b and c). The number of T cells markedly decreased in the thymus. The numbers of double-positive and CD4 single-positive thymocytes were more dramatically reduced, when compared with those of double-negative and CD8+ thymocytes (Fig. 4). These results indicated the higher sensitivity of double-positive and CD4+ thymocytes than of double-negative and CD8+ thymocytes in vivo. At day 7 after the injection of RK-8, thymic atrophy was evident since almost all thymocytes and other thymic cells disappeared (Fig. 3d). The damages was transient, because the thymus regenerated and had a normal constitution at 1 month after injection (Fig. 3e). Essentially the results were the same in the thymus of newborn mice (Fig. 2b; and Fig. 3f and g), except that recovery of the thymus could not be determined because newborn mice died within 7 days after injection of RK-8 (Table 1).

**Fig. 4.** Remarkable reduction of double-positive and CD4+ thymocytes by administration of RK-8. Control IgG (a) or RK-8 antibody (b) (5 mg/kg mouse) was i.p. injected into 6-week-old adult mice. The animals were sacrificed at day 1 after injection. Numbers of total viable thymocytes were counted, and the expression of CD4 and CD8 antigen on thymocytes was analyzed by cytofluorometry. Percentages and absolute numbers of double-negative, CD4+, CD8+ and double-positive thymocytes are also indicated. Absolute numbers were calculated by multiplying the total thymocytes number by the percentages of each subset.
Cytotoxicity of anti-Fas antibodies against hepatocytes in vitro. The i.p. administration of anti-Fas antibody Jo2 reportedly kills mice within 5 h (15). Histological analysis indicates severe focal hemorrhage and necrosis of the liver with Jo2. Our anti-Fas antibody RK-8 did not exert lethal effects in vivo, although the liver was focally damaged by apoptosis. To examine whether these antibodies had the same cytotoxic activity against hepatocytes, we analyzed the in vitro effect of Jo2 and RK-8 on primary cultures of hepatocytes. Hepatocytes were cultured with various concentrations of Jo2 or RK-8 for 20 h in the presence of 10 μg/ml cycloheximide and cell viability was examined. As shown in Fig. 1(c), hepatocytes were weakly, but significantly killed by Jo2 in a dose-dependent manner, although RK-8 had no detectable cytotoxicity against primary cultures of mouse hepatocytes.

Discussion
To examine the expression and function of Fas in mice, we prepared agonistic mAb against mouse Fas with associated apoptosis-inducing activity both in vitro and in vivo. Ogasawara et al. have reported that i.p. administration of the anti-mFas antibody (Jo2) into mice rapidly killed wild-type, but neither lpr nor lpr<sup>−</sup> mice, due to fulminant hepatitis with
Fig. 6. *In vivo* effect of RK-8 administration on the liver. RK-8 antibody (5 mg/kg mouse) was i.p. injected into 6-week-old adult mice (a–f). Animals were sacrificed before injection (a), and at day 1 (b, e), day 7 (c and f) and 1 month (d) after injection. The arrow indicates a hepatocyte with fragmented nucleus (e). RK-8 antibody or control IgG (5 mg/kg mouse) was s.c. injected into newborn mice (g and h), and the animals were sacrificed at day 5 after an injection of control IgG (g) and RK-8 (h). Liver sections were stained with azan (f) or hematoxylin & eosin (a–g). Magnification: ×20 (a and d), ×50 (b), ×33 (c, f, g and h) and ×100 (e).
it had no lethal effects on adult mice (Table 1). When RK-8 and Jo2 were i.v. or s.c. administered into adult mice, essentially the same results were obtained (data not shown). We have prepared eight kinds of specific anti-mFas mAb (9). RK-8 (hamster IgG) and RMF-2 (rat IgG1), both of which are specific to the allotype of Fas on BALB/c and MRL mice, show apoptosis-inducing activity both in vitro and in vivo, and another six kinds of mAb, i.e. C6-1, P4-4, SK-8 (hamster IgG), isoatypes of these mAb are unclear because isotype of hamster Ig has not been defined), RMF-6 (rat IgG2a), RMF-9 (rat IgG1) and RMF-13 (rat IgM), five of which are specific to the allotype of Fas on BALB/c and MRL (9), show no apoptosis-inducing activity both in vitro and in vivo (data not shown). Administration of RK-8 as well as RMF-2 into BALB/c mice caused severe damage of the thymus, and moderate damage of the spleen and liver. The in vivo effects of RK-8 and RMF-2 were not thought to be caused by either the complement-mediated cell lysis or antibody-dependent cellular cytotoxicity, because administration of other six kinds of our prepared hamster and studying the differentiation of hepatocytes and homeostasis of the liver (17), suggesting that soluble Fasl has characteristics intermediate between Jo2 and RK-8. The hepatic failure caused by RK-8 would be a good model of human chronic hepatitis, which can be caused by activation of the immune system components such as cytotoxic T cells.

RK-8 killed almost all the hepatocytes of newborn mice, but only some in the adults, indicating a different level of sensitivity to Fas-mediated apoptosis. Fas expression was reportedly elevated in human hepatocytes in hepatitis B virus-related cirrhosis and in acute liver failure (18). Thus, the characteristics of hepatocytes in hepatitis B virus-related cirrhosis and acute liver failure seem to resemble those of newborn mice. Those characteristics may be important when studying the differentiation of hepatocytes and homeostasis of the liver.

Cytfluorometric analyses have indicated that cells of the T lineage, except for c-kit+ immature cells, express Fas on the surface (9). CD4+CD8- undifferentiated thymocytes express low levels of Fas. Immature CD4+CD8+, as well as mature CD4+CD8- and CD4+CD8+ thymocytes are highly positive for Fas. CD4+CD8+ thymocytes are specifically sensitive to the apoptosis-inducing activity of anti-Fas in vitro, although CD4+CD8-, CD4+CD8- and CD4+CD8+ thymocytes are resistant (8–10). These results coincided with in vivo effects of RK-8, except for CD4+CD8+ thymocytes (Figs 3 and 4). The number of CD4+CD8- thymocytes was significantly decreased in thymus 1 day after an injection of RK-8, although these cells were resistant to apoptosis-inducing activity in vitro. Thus, the apoptosis-inducing activity of RK-8 in CD4+ thymocytes was stronger than that in CD8- thymocytes in vivo and the activity of RK-8 in vivo was stronger than that in vitro.

Histological examinations of thymus revealed that many thymocytes died by apoptosis 1 day after an injection of RK-8.
In vivo effects of anti-Fas mAb

(Fig. 3b and c). We suggested that these apoptotic cells were double-positive and CD4+ thymocytes, because they disappeared rapidly with the appearance of the apoptotic bodies in vivo. Not only double-positive and CD4+ thymocytes, but also almost all cells in the thymus including stroma cells, disappeared 1 week after an injection of RK-8 (Fig. 3c). Thus, RK-8 might cause apoptosis on not only CD4+CD8+ and CD4+CD8− thymocytes but also thymic stroma cells. Finally, RK-8 caused thymic atrophy, although the gland completely regenerated within 1 month after the administration of anti-Fas. These results suggested that the in vivo effect of RK-8 was transient, and that the stem or precursor cells for thymic lymphocytes and stroma cells were resistant to anti-Fas.

Some studies of lpr mice have suggested that there is a defect in the negative selection of self-reactive T cells in the thymus (19,20), although others have indicated that the Fas system is not involved in this process (21–23). We demonstrated the involvement of Fas in the negative selection/clonal deletion of human lymphocytes induced by staphylococcal enterotoxin B (24). Here we showed that mouse thymocytes at double-positive and CD4+ stages were extremely sensitive to apoptosis-inducing signals through Fas in vivo (Fig. 4). We suppose that the Fas system is at least partly involved in the negative selection/clonal deletion and/or differentiation of the mouse, as well as human lymphocytes, although the expression of Fas in the human thymus is quite different from that in the murine thymus (9,24).

Spleen T cells were resistant to anti-Fas in vitro, although they express Fas (9). Here we show that in vivo effect of RK-8 on spleen T cells was different from the in vitro effect. The administration of RK-8 reduced the number of spleen T cells to one-third of that in control mice and CD4+ spleen T cells were more sensitive to the apoptosis-inducing activity of anti-Fas than CD6+ T cells. CD4+ but not CD8+ spleen T cells might be directly killed by anti-Fas in vivo. Our results coincided with the reported data: peripheral T cells from lpr/lpr mice have a defect in the suicide pathway upon antigenic challenge both in vitro and in vivo (23) and Fas is involved in the suicide pathway of CD4+ but not CD8+ T cells (25). Fas is therefore responsible for the deletion of activated CD4+ mature T cells in vivo.

RK-8, which has powerful apoptosis-inducing activity both in vitro and in vivo, had no lethal effect on adult mice. RK-8 will allow the effect of Fas-mediated apoptosis on various diseases, including cancer and autoimmune disease, to be studied. We have started to analyze the ameliorative effects of RK-8 against autoimmune diseases in model mice.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and the Ministry of Health and Welfare, Japan.

Abbreviations

Fas Fas antigen
Fasl Fas ligand
gld generalized lymphoproliferative disease
GOT glutamic oxaloacetic transaminase
GPT glutamic pyruvic transaminase
lpr lymphoproliferation
mFas mouse Fas antigen

References

4 Yonehara, S., Ishii, A. and Yonehara, M. 1989. A cell-killing bodies in vivo. Not only double-positive and CD4+ thymocytes, and as human thymocytes, although the expression of Fas in the human thymus is quite different from that in the murine thymus (9,24).

Spleen T cells were resistant to anti-Fas in vitro, although they express Fas (9). Here we show that in vivo effect of RK-8 on spleen T cells was different from the in vitro effect. The administration of RK-8 reduced the number of spleen T cells to one-third of that in control mice and CD4+ spleen T cells were more sensitive to the apoptosis-inducing activity of anti-Fas than CD6+ T cells. CD4+ but not CD8+ spleen T cells might be directly killed by anti-Fas in vivo. Our results coincided with the reported data: peripheral T cells from lpr/lpr mice have a defect in the suicide pathway upon antigenic challenge both in vitro and in vivo (23) and Fas is involved in the suicide pathway of CD4+ but not CD8+ T cells (25). Fas is therefore responsible for the deletion of activated CD4+ mature T cells in vivo.

RK-8, which has powerful apoptosis-inducing activity both in vitro and in vivo, had no lethal effect on adult mice. RK-8 will allow the effect of Fas-mediated apoptosis on various diseases, including cancer and autoimmune disease, to be studied. We have started to analyze the ameliorative effects of RK-8 against autoimmune diseases in model mice.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and the Ministry of Health and Welfare, Japan.

Abbreviations

Fas Fas antigen
Fasl Fas ligand
gld generalized lymphoproliferative disease
GOT glutamic oxaloacetic transaminase
GPT glutamic pyruvic transaminase
lpr lymphoproliferation
mFas mouse Fas antigen

References

4 Yonehara, S., Ishii, A. and Yonehara, M. 1989. A cell-killing bodies in vivo. Not only double-positive and CD4+ thymocytes, and as human thymocytes, although the expression of Fas in the human thymus is quite different from that in the murine thymus (9,24).

Spleen T cells were resistant to anti-Fas in vitro, although they express Fas (9). Here we show that in vivo effect of RK-8 on spleen T cells was different from the in vitro effect. The administration of RK-8 reduced the number of spleen T cells to one-third of that in control mice and CD4+ spleen T cells were more sensitive to the apoptosis-inducing activity of anti-Fas than CD6+ T cells. CD4+ but not CD8+ spleen T cells might be directly killed by anti-Fas in vivo. Our results coincided with the reported data: peripheral T cells from lpr/lpr mice have a defect in the suicide pathway upon antigenic challenge both in vitro and in vivo (23) and Fas is involved in the suicide pathway of CD4+ but not CD8+ T cells (25). Fas is therefore responsible for the deletion of activated CD4+ mature T cells in vivo.

RK-8, which has powerful apoptosis-inducing activity both in vitro and in vivo, had no lethal effect on adult mice. RK-8 will allow the effect of Fas-mediated apoptosis on various diseases, including cancer and autoimmune disease, to be studied. We have started to analyze the ameliorative effects of RK-8 against autoimmune diseases in model mice.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and the Ministry of Health and Welfare, Japan.

Abbreviations

Fas Fas antigen
Fasl Fas ligand
gld generalized lymphoproliferative disease
GOT glutamic oxaloacetic transaminase
GPT glutamic pyruvic transaminase
lpr lymphoproliferation
mFas mouse Fas antigen

References

4 Yonehara, S., Ishii, A. and Yonehara, M. 1989. A cell-killing bodies in vivo. Not only double-positive and CD4+ thymocytes, and as human thymocytes, although the expression of Fas in the human thymus is quite different from that in the murine thymus (9,24).

Spleen T cells were resistant to anti-Fas in vitro, although they express Fas (9). Here we show that in vivo effect of RK-8 on spleen T cells was different from the in vitro effect. The administration of RK-8 reduced the number of spleen T cells to one-third of that in control mice and CD4+ spleen T cells were more sensitive to the apoptosis-inducing activity of anti-Fas than CD6+ T cells. CD4+ but not CD8+ spleen T cells might be directly killed by anti-Fas in vivo. Our results coincided with the reported data: peripheral T cells from lpr/lpr mice have a defect in the suicide pathway upon antigenic challenge both in vitro and in vivo (23) and Fas is involved in the suicide pathway of CD4+ but not CD8+ T cells (25). Fas is therefore responsible for the deletion of activated CD4+ mature T cells in vivo.

RK-8, which has powerful apoptosis-inducing activity both in vitro and in vivo, had no lethal effect on adult mice. RK-8 will allow the effect of Fas-mediated apoptosis on various diseases, including cancer and autoimmune disease, to be studied. We have started to analyze the ameliorative effects of RK-8 against autoimmune diseases in model mice.

Acknowledgements

This work was supported in part by grants from the Ministry of Education, Science and Culture of Japan and the Ministry of Health and Welfare, Japan.

Abbreviations

Fas Fas antigen
Fasl Fas ligand
gld generalized lymphoproliferative disease
GOT glutamic oxaloacetic transaminase
GPT glutamic pyruvic transaminase
lpr lymphoproliferation
mFas mouse Fas antigen

References

In vivo effects of anti-Fas mAb


