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Fas-induced *in vivo* Apoptosis in Bone Marrow: Anti-Fas mAb-induced Elimination and Successive Proliferation of Fas-expressing Cells Especially Those of Myeloid Lineage

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ABSTRACT. **A single administration of agonistic anti-Fas mAb RK8 into mice decreased the number of bone marrow cells especially Mac1$^+$ and Gr1$^+$ cells of myeloid lineage. These cells, which were shown to be Fas-positive in normal bone marrow, were directly eliminated *in vivo* by Fas-mediated apoptosis. After the elimination of Fas-positive bone marrow cells, bone marrow was reconstituted by successive increase of numbers of Gr1^{low} and Mac1^{low} myeloid precursor cells expressing high levels of Fas, which are minor constituents in normal bone marrow. The increased cells consisted at least two components, Gr1^{dull} Mac1$^+$ cKit$^+$ cells and Gr1^{intermediate} Mac1$^+$ cKit$^-$ cells, both of which were shown to be sensitive to Fas-induced apoptosis *in vivo*. Thus, Fas is functional in normal bone marrow and Fas-induced apoptosis in bone marrow enhances marked proliferation of Fas-expressing myeloid precursor cells *in vivo*.**

Key words: hematopoiesis/granulocyte/cell death/flow cytometry/GVHD (graft-versus-host disease)

Proliferation and differentiation of hematopoietic progenitor cells are known to occur in bone marrow with the help of cytokines and/or cell-to-cell interactions with stroma cells (1, 2). Not only proliferation and differentiation but also apoptosis of hematopoietic progenitor cells are reportedly induced *in vitro* by the depletion of growth-promoting cytokines from the culture medium (3, 4). Fas is a cell surface receptor molecule belonging to the TNF receptor family (5) which can introduce apoptosis-inducing signals into Fas-expressing cells upon stimulation with Fas ligand (FasL) or agonistic anti-Fas mAb (5, 6). Expression of Fas on bone marrow cells was reportedly very weak or undetectable (7–9) and was shown to be induced *in vitro* by stimulation with inflammatory cytokines such as IFN and TNF (10, 11). Recently, Traver *et al.* reported the expression of Fas on bone marrow cells in the myeloid lineage (12). It has not been shown, however, whether the stimulation of Fas can induce apoptosis in bone marrow cells *in vivo*, although the cytokine-induced Fas could reportedly mediate apoptosis-inducing signals in bone marrow-derived cells *in vitro* (10).

FasL was shown to express mainly on activated T and NK cells, which play an important role in the elimination of self-reactive immunocytes, tumor cells and viral infected cells (5, 13). In the case of MHC class II-restricted graft-versus-host disease (GVHD), in which graft-derived activated CD4$^+$ T cells play an important role, FasL on the T cells was shown to induce apoptosis in host cells (14, 15). Recently, Mori *et al.* reported that FasL plays a role in myelosuppression of GVHD (16). Fas might be involved in the loss of hematopoietic cells in GVHD. Interestingly, mutations of Fas gene were shown in patients with multiple myeloma (17) and Fas-mediated cell death pathway is reportedly altered in malignant cells from non-Hodgkin's lymphoma (18). Recently, Traver *et al.* reported that Fas-deficient *lpr/lpr* mice constitutively expressing Bcl-2 in myeloid cells often develop a fatal disease similar to acute myeloblastic leukemia (12). These results suggest an important role for Fas-FasL system-induced apoptosis in the elimination of hematopoietic tumor cells *in vivo*. Thus, we suspected that Fas induces apoptosis *in vivo* in hematopoietic progenitor cells in bone marrow.

We have prepared agonistic hamster anti-mouse Fas

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Abbreviations: FasL, Fas ligand; GVHD, graft-versus-host disease; PE, phycoerythrin; PI, propidium iodide.

mAb RK8 (9) which can induce apoptosis both *in vitro* and *in vivo*, and analyzed the *in vivo* effects of RK8 (19, 20). Interestingly, a single administration of RK8 did not kill adult mice, although mice given another agonistic anti-mouse mAb, Jo-2, rapidly died of fulminant hepatitis with hemorrhage (19, 21). Histological analyses of mice given RK8 indicated the induction of *in vivo* apoptosis in cells expressing Fas, such as thymocytes, hepatocytes and CD4 positive spleen T cells (19), granulosa and lutein cells in ovary (22), *gld* cells in lymphoadenopathy (20) and synovial cells in articular rheumatism (20, 23). In this study, we demonstrated the induction of *in vivo* apoptosis and successive proliferation of bone marrow cells, especially those of immature cells in granulocyte and monocyte/macrophage lineages, on RK8 administration.

Materials and Methods

Mice

C57B/6J-+/+, C57B/6J-*lpr/lpr* and Balb/c mice were purchased from Japan SLC Inc. (Shizuoka, Japan).

Administration of antibodies

Purified agonistic hamster anti-mouse Fas mAb RK8 (100 μg/mouse) was intraperitonealy injected into Balb/c male mice as described previously (19, 20). The same amounts of control hamster IgG (ICN Pharmaceuticals, Aurora, OH, USA) were similarly administered to control mice. At various days after injection, mice were sacrificed by euthanasia with anesthetic, and bone marrow cells were isolated. The freshly isolated cells were analyzed by flow cytometry and the number of bone marrow cells was counted after staining with Turk's stain solution (Nacalai Tesque, Kyoto, Japan).

Flow cytometry

Abs used in this work were FITC-conjugated TER119 mAb (a gift from Dr. Tatsuo Kina, Kyoto University, Japan), phycoerythrin (PE)-anti cKit mAb (a gift from Dr. Shinichi Nishikawa, Kyoto University, Japan), FITC-conjugated B220 mAb (Pharmingen, San Diego, CA, USA), FITC- or PE-conjugated Mac1 mAb (Cedarlane, Westbury, NY, USA), FITC-conjugated Gr1 mAb (Cedarlane), PE-conjugated hamster anti-mouse Fas mAb Jo2 (Pharmingen) and PE-conjugated polyclonal hamster IgG isotype standard (Pharmingen). The bone marrow cells were incubated on ice for 40 min in staining buffer (PBS containing 5% FBS and 0.05% NaN_3) containing various Abs with 1 μg/ml propidium iodide (PI). Two-color flow cytometry analyses and cell sorting were performed using an EPICS^R Elite (Coulter, Fullerton, CA, USA).

DNA fragmentation assay

Cellular DNA was isolated and fractionated by electrophoresis in 2% agarose gel as previously described (24). DNA was visualized by staining with SYBR^{TM} Green I (Wako Pure Chemical Industries, Osaka, Japan).

Results

In vivo effects of agonistic anti-Fas mAb on bone marrow cells

To investigate the biological significance of Fas in bone marrow, 100 μg/mouse of agonistic hamster anti-mouse Fas mAb RK8 was intraperitonealy injected into 3 week-old Balb/c male mice. This dose of RK8 is enough to induce apoptosis in thymocytes *in vivo* without killing the mouse (19). The bone marrow cells were isolated at various days after injection and the isolated cells were immediately analyzed by flow cytometry.

We performed flow cytometric analyses for bone marrow cells from RK8-injected mice after gating out the PI-positive cells in order to eliminate dead cells. Figure 1A shows RK8-induced remarkable decrease and successive increase of the percentage of $Gr1^+$ and $Mac1^+$ cells. One day after injection, the percentages of $Gr1^+$ and $Mac1^+$ cells were half of those before injection. Then, both $Gr1^+$ and $Mac1^+$ cells increased in number from day 2 to day 4 after injection and they decreased by day 10. In contrast with $Gr1^+$ cells and $Mac1^+$ cells, decrease of the percentage of $B220^+$ cells was not observed 1 day after injection. $B220^+$ cells, however, decreased in number from day 4 to day 7 after injection. Then, the number of $B220^+$ cells increased and reached normal level at day 10. On the contrary, remarkable change of $TER119^+$ cells could not be detected from day 0 through day 10. The total number of cells in a femur did not significantly change from day 0 through day 10 (data not shown). These *in vivo* effects of RK8 on bone marrow are RK8-specific because mice injected with control hamster Ig showed no significant changes in bone marrow (data not shown).

To determine whether RK8 induces apoptosis in bone marrow cells *in vivo*, DNA fragmentation was quantified. Figure 1B shows DNA fragmentation in bone marrow cells 1 day and 2 days after injection, although a DNA ladder was undetectable in bone marrow cells at day 0 and day 4 after injection. These results indicate that apoptosis was induced in bone marrow cells *in vivo* 1 day and 2 days after injection of RK8.

Expression of Fas on freshly isolated bone marrow cells

Although we showed here anti-Fas mAb-induced *in*

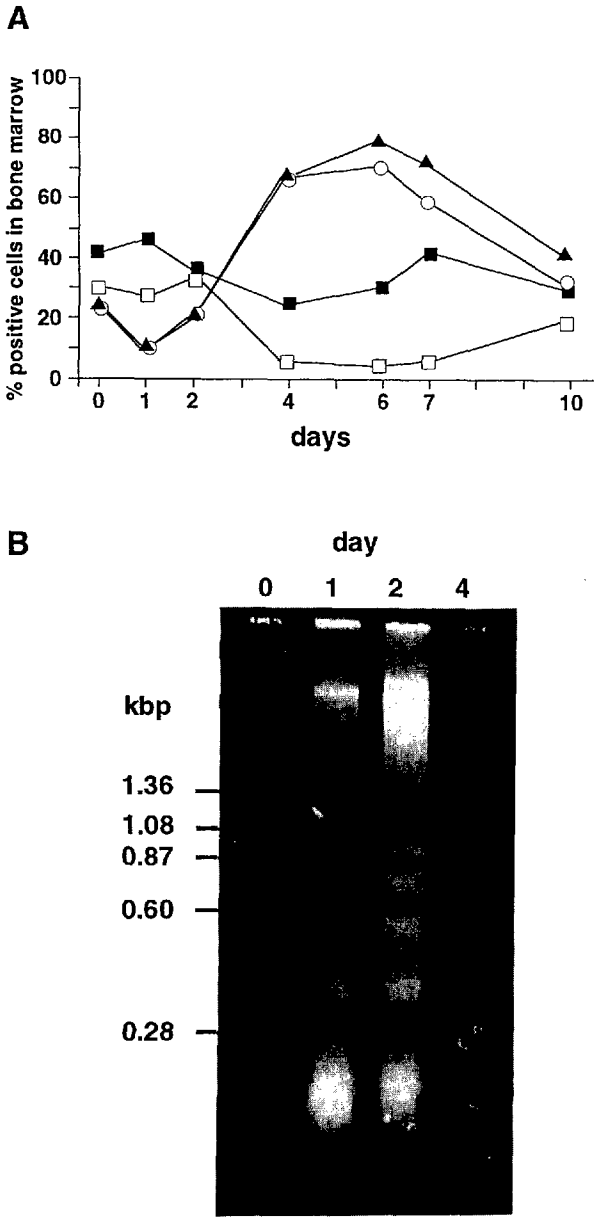


Fig. 1. Effects of anti-Fas mAb on bone marrow cells. Balb/c mice were injected with 100 μg/mouse RK8 and the isolated bone marrow cells were analyzed by flow cytometry at day 0, 1, 2, 4 and 10 after injection. (A) The percentages of Mac1$^+$ (triangle), Gr1$^+$ (circle), TER119$^+$ (filled square) and B220$^+$ (opened square) cells in bone marrow were determined using a flow cytometer with gating out of PI-positive cells. (B) DNA fragmentation was analyzed on bone marrow cells as described in *Materials and Methods* at day 0, 1, 2 and 4 after RK8 injection.

vivo apoptosis in bone marrow cells, previous reports indicated that the expression of Fas on bone marrow cells was very weak or undetectable (7–9). Recently, however, Traver *et al.* reported the expression of Fas on bone marrow cells in myeloid lineage (12). Here, we

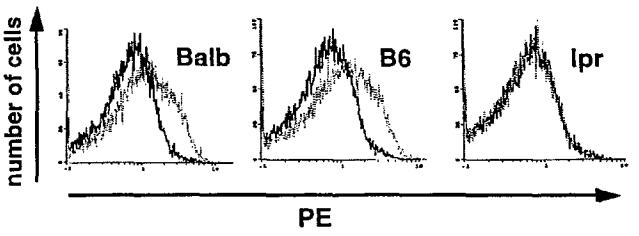


Fig. 2. Fas-expression on bone marrow cells. The bone marrow cells from 3 week-old Balb/c male mice (Balb), C57B/6J male mice (B6) and C57B/6J-*lpr/lpr* (lpr) were stained with control hamster IgG-PE (solid line) or anti-Fas mAb Jo2-PE (dash line), and analyzed by flow cytometry after gating out PI-positive cells. The experiment is representative of three similar experiments.

analyzed the expression of Fas on bone marrow cells from 3 week-old Balb/c male mice, and 3 week-old C57B/6J male mice, and these cells were shown to express Fas (Fig. 2). Expression of Fas on these bone marrow cells was significant because we did not detect Fas expression on bone marrow cells of 3 week-old C57B/6J-*lpr/lpr* mice which do not express Fas (25). We analyzed the expression of Fas on bone marrow cells of 6 week-old Balb/c male mice (data not shown), and essentially similar results were obtained as for 3 week-old mice.

Expression of Fas on bone marrow cells after in vivo administration of RK8

Administration of RK-8 induced the decrease and successive increase in the number of Gr1$^+$ cells and Mac1$^+$ cells (Fig. 1A). We then analyzed the expression of Fas together with lineage markers on bone marrow cells after RK8 injection.

One day and two days after injection, the number of Fas-positive bone marrow cells decreased (data not shown) and then increased remarkably. Figure 3 shows the results for bone marrow cells from mice before injection (day 0) and 4 days after injection. Before RK8 injection, Fas was expressed significantly on the part of TER119$^-$ cells, B220$^-$ cells and Mac1$^+$ cells. Weak expression of Fas was observed on Gr1^{low} cells and little expression on Gr1^{high} cells was detected. From 2 days after RK8 injection, TER119$^-$ cells and B220$^-$ cells expressing Fas increased in number. Interestingly, Gr1^{low} cells, which strongly expressed Fas, proliferated markedly. The Gr1^{low} cells could be divided into Gr1^{dull} and Gr1^{intermediate} (Gr1^{int}) cells, which express lower and higher amounts of Gr1, respectively, when compared with the other constituent of Gr1^{low} cells (Fig. 3). The number of Gr1^{high} cell also increased 4 days after injection, although the expression level of Fas on Gr1^{high} cells was very weak. Mac1$^+$ cells, composed of Mac1^{low} and Mac1^{high} cells, also proliferated. Mac1^{low}

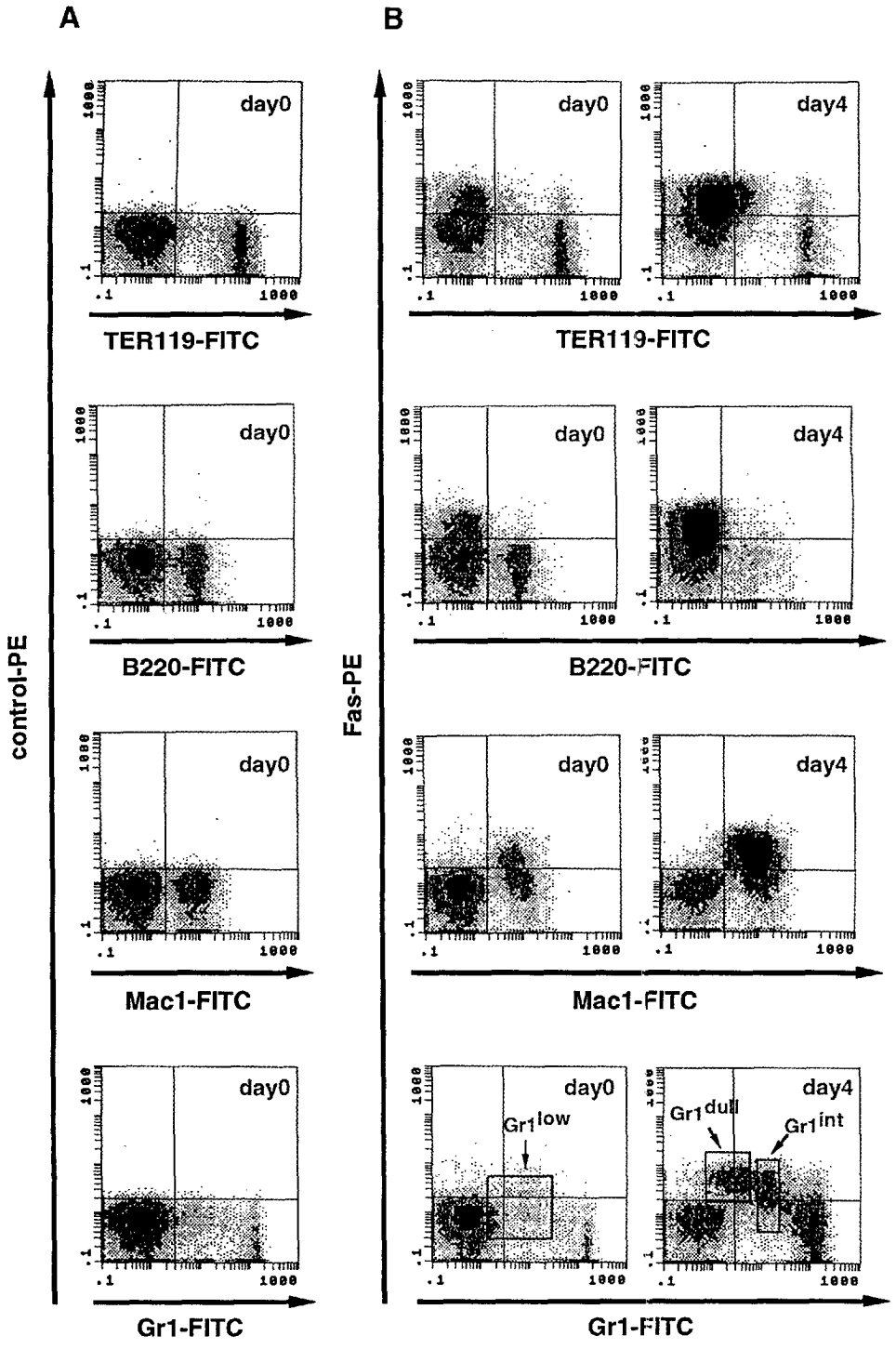
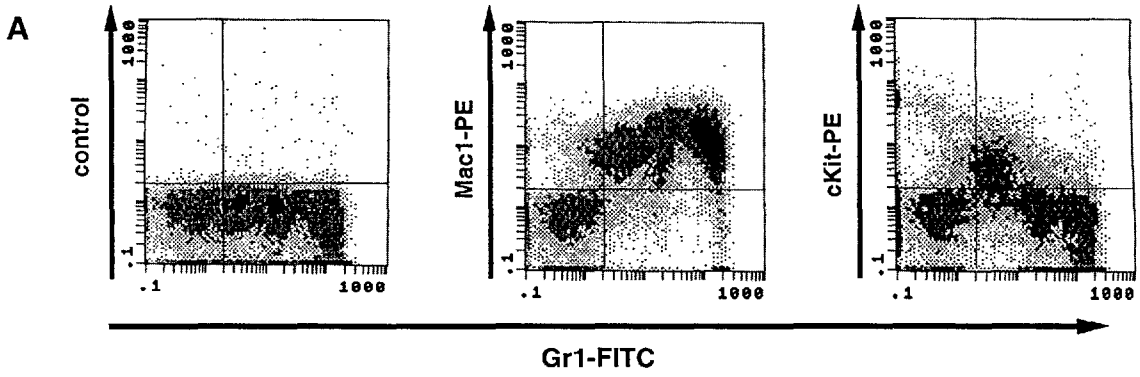


Fig. 3. Flow cytometric analyses of bone marrow cells after injection of RK8. Balb/c mice were injected with 100 μg/mouse RK8 and the bone marrow cells isolated at day 0 and day 4 after injection were stained with control hamster IgG-PE (A) or anti-Fas mAb-PE (B) together with TER119-FITC, B220-FITC Mac1-FITC or Gr1-FITC in the presence of PI. After gating out PI-positive cells, expression levels of these antigens were analyzed. The experiment is representative of five similar experiments.

cells, which were hardly detectable in normal bone marrow, strongly express Fas. Thus, the increased populations at day 4 after injection seems to be essentially the same as those expressing Fas in normal bone marrow although the number of these Fas-positive cells is few in normal bone marrow.

Fas-positive cells in bone marrow were shown to disappear at 1 day or 2 days after injection (data not shown), and then to remarkably increase in number. The increased-populations with Fas which contain $Gr1^{low}$ cells and $Mac1^{low}$ cells were minor constituents of normal bone marrow, and might be precursor cells of myeloid lineage as reported previously (26). These observations suggest that Fas-mediated elimination of



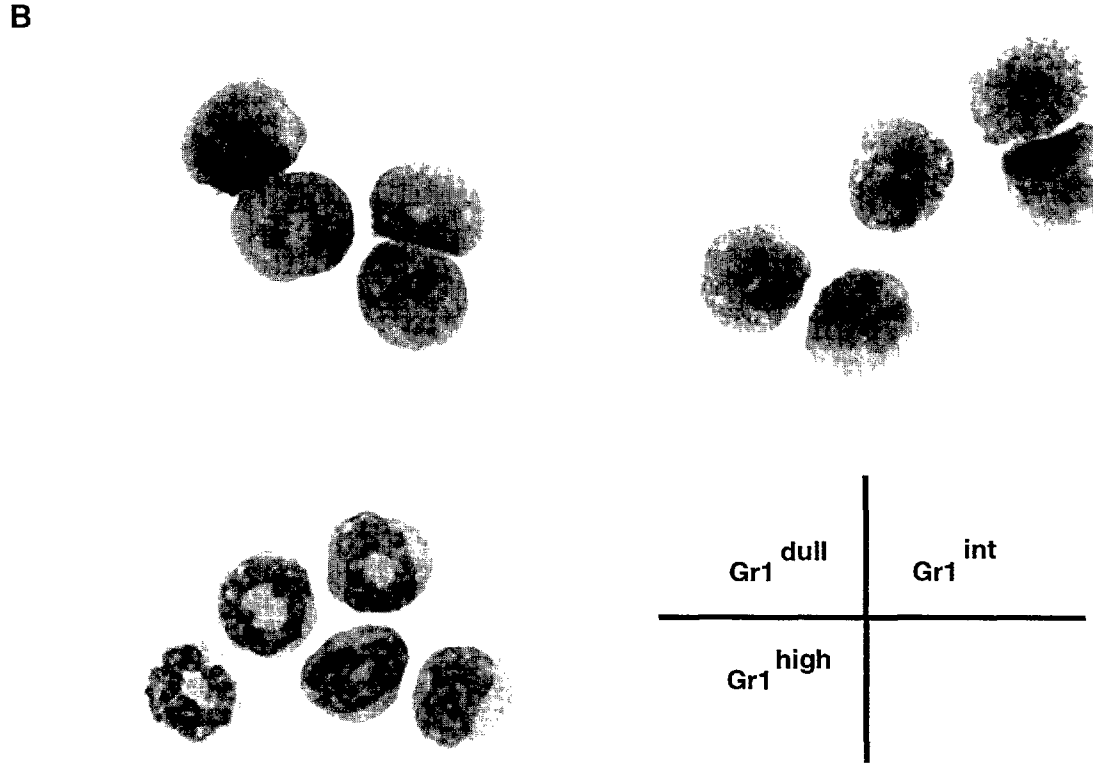


Fig. 4. Analysis of Gr1-expressing bone marrow cells after injection of RK8. (A) Flow cytometric analysis. Balb/c mice were injected with 100 μg/mouse RK8 and the bone marrow cells isolated at day 4 after injection were stained with Abs described in the figure. (B) Morphological analysis of bone marrow cells after injection of RK8. Four days after RK8 administration, $Gr1^{dull}$, $Gr1^{int}$ and $Gr1^{high}$ cells from the bone marrow were separately isolated by cell sorter (EPICS^{R} Elite). The sorted cells were collected on slide glass by cytocentrifugation, stained with Wright's stain solution (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) and observed under a light microscope. The experiment is representative of four similar experiments.

bone marrow cells excessively induces successive proliferation and differentiation of hematopoietic progenitor cells to compensate for the eliminated cells.

Analyses of the reconstituted bone marrow cells expressing Fas and Gr1

Four days after RK8 injection, the number of $Gr1^+$ cells, especially $Gr1^{low}$ ($Gr1^{dull}$ and $Gr1^{int}$) cells in bone marrow remarkably increased (Fig. 3). Then, the number of both of $Gr1^{dull}$ cells and $Gr1^{int}$ cells began to decrease, and 10 days after injection, the number reached the same level as before (data not shown). These observations suggest that these $Gr1^{dull}$ and $Gr1^{int}$ cells are progenitor cells in the myeloid lineage. We analyzed the expression of cKit and Mac1 on these $Gr1^+$ cells because immature myeloid cells reportedly express cKit and Mac1 (26). Figure 4A shows that cKit is expressed on $Gr1^{dull}$ cells but not on $Gr1^{int}$ cells or $Gr1^{high}$ cells. Mac1 is expressed on most of $Gr1^+$ cells. Morphological analyses of these $Gr1^{dull}$, $Gr1^{int}$ and $Gr1^{high}$ cells were then performed after these populations were sorted by a flow cytometer. Figure 4B shows that $Gr1^{dull}$ cells consist of myeloid precursor cells with round uncondensed nuclei and basophilic cytoplasms; $Gr1^{int}$ cells consist of slightly differentiated cells with a little condenced nuclei and many granules in the cytoplasm which appear to be primary granules; and $Gr1^{high}$ cells consist of fairly differentiated cells with ring-shaped nuclei and secondary granules in cytoplasms. These results indicate that $Gr1^{dull}$ cells are more immature than $Gr1^{int}$ cells while $Gr1^{int}$ cells are still immature, and $Gr1^{high}$ cells are mature myeloid cells.

$Gr1^{dull}$ and $Gr1^{int}$ cells express Fas (Fig. 3B). To investigate whether these immature myeloid cells are sensitive or insensitive to Fas-mediated apoptosis, we again injected RK8 into mice given RK8 4 days before. Figure 5A shows that the number of $Mac1^+$ cells decreased by the second injection of RK8. Both $Gr1^{dull}$ $Mac1^+$ $cKit^+$ cells and $Gr1^{int}$ $Mac1^+$ $cKit^-$ cells, which express Fas, were eliminated by the second administration, although $Gr1^{high}$ cells remained (Fig. 5B).

To determine whether the decrease of $Mac1^+$ and

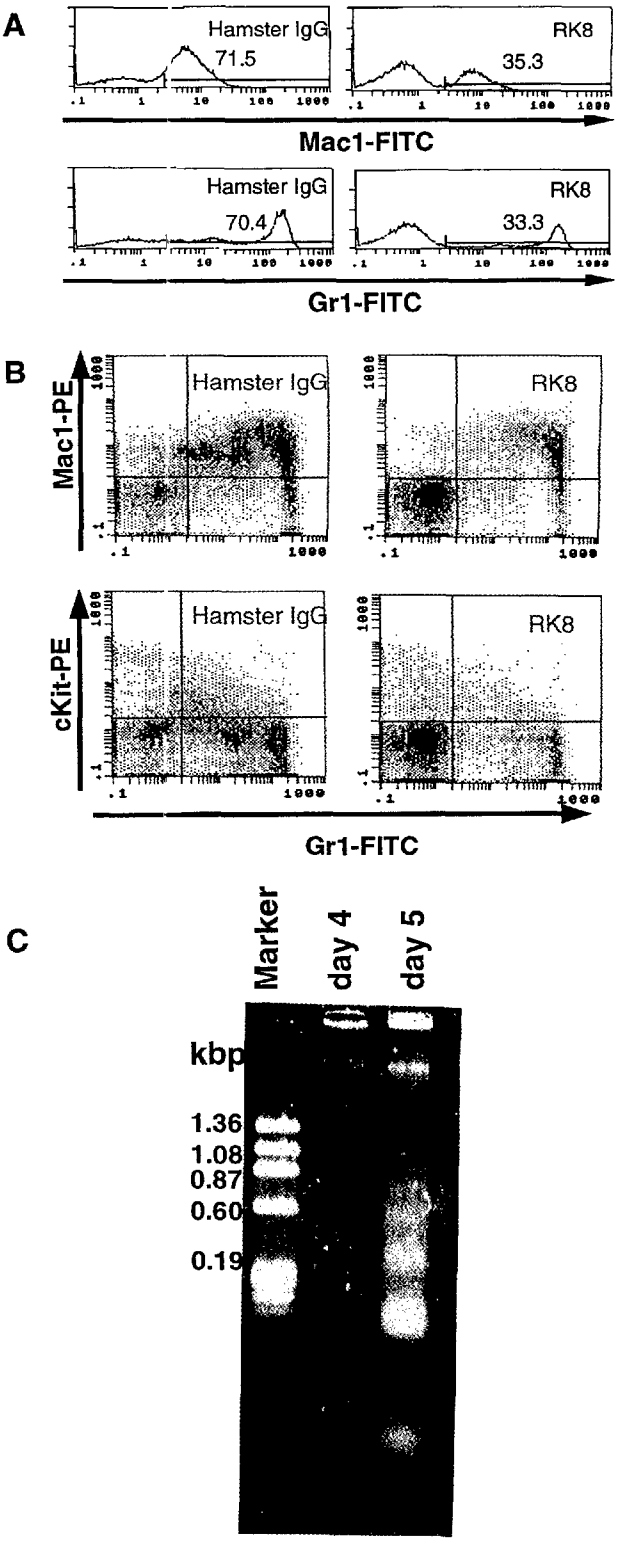


Fig. 5.

Fig. 5. Effects of repeated administration of RK8 on bone marrow cells. Balb/c mice, given 100 μg/mouse RK8, were injected again with 100 μg/mouse RK8 (indicated as RK8 in the figure) or control hamster IgG (indicated as hamster IgG in the figure) 4 days after the first administration of RK8. (A) Bone marrow cells one day after the second injection were analyzed by flow cytometry after being stained with Mac1-FITC or Gr1-FITC. Percentages of Mac1-positive and Gr1-positive cells were indicated. (B) Bone marrow cells one day after the second injection were analyzed by two-color flow cytometry after being stained with Gr1-FITC and Mac1-PE, or Gr1-FITC and cKit-PE. (C) DNA fragmentation was analyzed in bone marrow cells before (day 4) and one day after the second injection of RK8 (day 5). The experiment is representative of four similar experiments.

$Gr1^+$ cells by the second RK8 injection was caused by apoptosis, DNA fragmentation was quantified. One day after the second RK8 injection, DNA fragmentation was detected in bone marrow cells, although significant DNA ladder was undetectable before the second injection (Fig. 5C). These observations show that Fas-mediated apoptosis can be induced on Fas expressing cells 4 days after RK8 injection, which consist of $Gr1^{dull}$ $Mac1^+$ $cKit^+$ and $Gr1^{int}$ $Mac1^+$ $cKit^-$ cells.

Discussion

In vivo Fas-induced apoptosis in bone marrow

Fas-mediated *in vivo* apoptosis has not been directly shown in hematopoietic cells in bone marrow, although Fas-mediated *in vitro* apoptosis was reported in these cells after cultivation with inflammatory cytokines (10, 11). In this study, we demonstrated that administration of agonistic anti-mouse Fas mAb RK8 decreased the number of bone marrow cells especially those of the granulocyte ($Gr1^+$) lineage and monocyte/macrophage ($Mac1^+$) lineage, and that DNA fragmentation was simultaneously induced in bone marrow cells. In addition, the decreased cells were shown to express Fas in normal bone marrow. These results indicate the direct induction of apoptosis in these bone marrow cells *in vivo* by the stimulation of Fas.

Reconstitution of bone marrow after the induction of apoptosis

We suspected that bone marrow is reconstituted after the *in vivo* elimination of Fas-positive cells by apoptosis. Actually the number of $Mac1^+$ cells and $Gr1^+$ cells began to increase remarkably from day 2 after administration of RK8. Figure 3B shows dramatic increase of $Gr1^{low}$ cells which are composed of at least two populations, $Gr1^{dull}$ and $Gr1^{int}$. Expression level of Gr1 on $Gr1^{dull}$ cells is lower than that of $Gr1^{int}$ cells. These $Gr1^{low}$ cells clearly appeared at day 2 after RK8 administration (data not shown). $Gr1^{dull}$ cells, which may be more immature than $Gr1^{int}$ cells, seem to differentiate to $Gr1^{int}$ cells and then to $Gr1^{high}$ cells because $Gr1^{dull}$ cells which express cKit, an immature marker of hematopoietic cells, appeared more rapidly than $Gr1^{int}$ cells after RK8 injection (data not shown). Interestingly, $Gr1^{dull}$ $Mac1^+$ $cKit^+$ cells, suggest that the cells are immature cells which can differentiate into either granulocytes or monocytes. In addition, morphological analyses also indicated that $Gr1^{dull}$ and $Gr1^{int}$ cells are more immature myeloid cells than $Gr1^{int}$ and $Gr1^{high}$ cells, respectively (Fig. 4C). Ten days after injection, decrease of the $Gr1^{low}$ $Mac1^{low}$ cells, and simultaneous increase of $Gr1^{high}$ $Mac1^{high}$ cells were observed (data not shown). These observations suggest that $Gr1^{dull}$ $Mac1^{low}$ $cKit^+$ cells are progenitor cells of myeloid lineage as reported in IL-2-deficient mice (26).

After the elimination of bone marrow cells especially those of the myeloid lineage by Fas-induced apoptosis, myeloid progenitor cells which express high level of Fas and low levels of Gr1 and Mac1 successively increased in number. These immature myeloid cells continued to proliferate from day 2 to day 4 after injection. Surprisingly, the number of $Gr1^+$ and $Mac1^+$ cells at day 4 after injection was about 3-fold more than that before injection. Such overproliferation might be the natural response of bone marrow for the elimination of myeloid progenitor cells by apoptosis within bone marrow, although another possibility remains that other *in vivo* effects of RK8, such as elimination of Fas^+ T cells or hepatocytes, induce the proliferation of bone marrow cells.

The proliferated immature $Gr1^{low}$ cells were shown to differentiate into mature $Gr1^{high}$ or $Mac1^{high}$ cells. These mature cells might leave the bone marrow until day 10 after injection, since the number of $Mac1^+$ cells and $Gr1^+$ cells decreased to normal level 10 days after injection (Fig. 1A). Thus, $Gr1^+$ cells and $Mac1^+$ cells reconstituted bone marrow after the induction of apoptosis by the stimulation of Fas *in vivo*. Surprisingly, the total cell number in a femur did not change significantly from day 0 though day 10 after RK8 injection (data not shown), suggesting that the total cell number in a femur is strictly fixed even if apoptosis and successive proliferation are induced in hematopoietic cells in bone marrow.

Apoptosis was induced in bone marrow cells by the second administration of RK8, at day 4 after the first injection (Fig. 5C), suggesting that the $Gr1^{low}$ cells expressing Fas in bone marrow, which were shown to proliferate from day 2 after the first administration of RK8, are sensitive to Fas-mediated apoptosis. However, apoptosis was not detected in these proliferated $Gr1^{low}$ cells without the second administration of RK8. These results show that Fas ligand, which can induce apoptosis in Fas-expressing cells, is absent in bone marrow. The expression of Fas in myeloid precursor cells, however, was indicated to be physiologically significant, since Fas reportedly acts as tumor suppressor to control leukemogenic transformation in myeloid progenitor cells (12, 17).

From 2 days after injection, $B220^+$ cells, which did not express Fas (Fig. 3B), began to decrease in number although the decrease in the number of $B220^+$ cells was not observed 1 day after injection (Fig. 1A). These observations suggest that the increase of $Gr1^+$ cells and $Mac1^+$ cells from 2 days to 4 days after injection might indirectly inhibit proliferation of $B220^+$ cells. On the contrary, we could not detect significant decrease or in-

crease in the number of TER119-positive cells after the stimulation of Fas *in vivo* (Fig. 1A). The cell number of erythroid lineage may be controlled more tightly in bone marrow than that of B cell lineage. Some of the bone marrow cells of erythroid lineage, however, might be eliminated by Fas-mediated apoptosis since a part of $TER119^+$ cells significantly express Fas (Fig. 3B). The remarkable decrease and successive increase of myeloid lineage, whereas the number of B cells ($B220^+$ cells) in bone marrow were dramatically affected by the increase of myeloid precursor cells.

Fas expression on hematopoietic cells in bone marrow

In this study, significant expression of Fas was observed on normal bone marrow cells, especially on $Gr1^{low}$ myeloid precursor cells whereas Fas was hardly detected on most of the $Gr1^{high}$ cells (Fig. 2, 3 and 5B). On the contrary, Traver *et al.* reported they detected Fas expression on $Gr1^{high}$ cells in normal bone marrow (12), which might be more differentiated cells than $Gr1^{low}$ cells. We suppose that Fas-expression on $Gr1^{low}$ cells is more essential than on $Gr1^{high}$ cells, because *in vivo* stimulation of Fas by RK8 induced elimination and successive proliferation of $Gr1^{low}$ myeloid progenitor cells but not $Gr1^{high}$ cells.

Physiological role of Fas in bone marrow

To analyze the physiological role of Fas in bone marrow, we compared the constituents of bone marrow ($B220^+$, $TER119^+$, $Gr1^+$ and $Mac1^+$ cells) in *lpr/lpr* mice with those in wild type mice. However, we could not detect significant difference between bone marrow cells from *lpr/lpr* and wild type mice (data not shown). On the other hand, mutations of Fas gene were recently reported in bone marrow cells of patients with multiple myeloma (17), suggesting the important role of Fas-FasL system-induced apoptosis in the elimination of hematopoietic tumor cells *in vivo*. Actually, Traver *et al.* recently reported that Fas-deficient *lpr/lpr* mice constitutively expressing Bcl-2 in myeloid cells often develop a fatal disease analogous to human acute myeloblastic leukemia (12). These reports suggest that Fas plays physiological roles in the elimination of hematopoietic tumor cells. In addition, hematopoietic suppression was reported in human hematopoietic bone marrow cells after the induction of Fas by the treatment with inflammatory cytokines *in vitro* suggesting that activated hematopoietic precursor cells in bone marrow might be eliminated by the Fas/FasL system (10), although we could not detect any significant difference between bone marrow cells from *lpr/lpr* and wild type mice.

In this study, we show that a single administration of anti-Fas mAb RK8 into mice induces transient loss of bone marrow cells of granulocyte and monocyte/macrophage lineages, and successive increase of myeloid precursor cells expressing Fas. We suspect that severe damage of bone marrow would be induced if the Fas-FasL system is continuously stimulated in bone marrow, because the cells, which proliferate and recover the normal construction of bone marrow after stimulation of Fas, express high levels of functional Fas which can transduce apoptosis-inducing signals (Fig. 3B and 5C). Such continuous stimulation by FasL must be induced on GVHD since Fas-mediated apoptosis by FasL on graft-derived activated $CD4^+$ T cells is reportedly involved in GVHD (13–15). Recently, Mori *et al.* reported that administration of an anti-FasL Ab with neutralizing activity might prevent myelosuppression induced in GVHD (16). In GVHD, hematopoietic damage could be directly induced by the Fas-FasL system in bone marrow.

In this report, we showed the expression of functional Fas which can mediate apoptosis-inducing signals, on bone marrow cells especially those of granulocyte and monocyte/macrophage lineages. Interestingly, administration of agonistic anti-Fas mAb RK8 was shown to induce apoptosis in these Fas^+ bone marrow cells and successive increase of Fas^+ myeloid progenitor cells. The physiological roles of Fas in these bone marrow cells require further investigation.

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