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Induction of Apoptosis in CD34$^+$ Cells by Sera from Patients with Aplastic Anemia

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ABSTRACT

To investigate the pathogenesis of aplastic anemia (AA), the apoptosis of CD34$^+$ cells was assayed with annexin V-fluorescein 24hr and 48hr after incubation with the serum of normal controls (n=10), patients with non severe AA (NSAA, n=13) and severe AA (SAA, n=10). The CD34$^+$ progenitors from the bone marrow of normal donors contained a significantly greater proportion of apoptotic cells after incubation with serum from SAA patients than that incubated with serum from normal controls and NSAA. Moreover, the percent apoptosis of CD34$^+$ cells after 24hr incubation with serum from NSAA was slightly higher than that of normal controls. This appears to be related to clinical severity. No significant difference was found in the percent apoptosis of CD34$^+$ cells between incubation for 24hr and 48hr with the same serum. To further explore the mechanism of increased apoptosis induced by the serum of AA patients, the expression of the Fas receptor was measured after incubation with serum from the normal controls and AA patients using flow cytometry. After incubation with serum from the AA patients, the Fas receptor was overexpressed, correlating with the increased apoptosis induced by the same serum from AA patients. In addition, the induction of apoptosis and Fas expression on CD34$^+$ cells by serum from the SAA patients was blocked partly by preincubation of the serum with anti-γ-IFN neutralizing MoAb. These findings suggested that some aberrant components of the serum in the AA patients, which was confirmed partly to be γ-IFN, can induce CD34$^+$ progenitors apoptosis through the Fas signaling pathway. This may contribute to understanding the decreased number of stem cells characteristic of aplastic anemia.

Key words: *Aplastic anemia, Apoptosis, Fas, Serum*

Aplastic anemia (AA) is characterized by peripheral blood pancytopenia and bone marrow hypocellularity. Recent reports have indicated that apoptosis plays an important role in the injury to the stem and progenitor compartments in these patients. Maciejewski et al have demonstrated an increased expression of Fas receptor (Fas-R) in CD34$^+$ cells from patients with AA[11]. Philpott et al have shown that the subset of CD34$^+$ cells from these patients contained a greater proportion of apoptotic cells than normal CD34$^+$ bone marrow cells[15]. In addition, Callera et al showed a significant increase in the proportion of mononuclear apoptotic cells in paraffin embedded bone marrow biopsies from patients with aplastic anemia[2]. However, little is known about the mechanism.

In a recent study, we demonstrated the inhibiting activity of serum on hematopoiesis in patients with SLE and in patients with aplastic anemia[8,9]. We also revealed that the hematopoietic suppression caused by serum from patients with AA was associated with aberrant γ-IFN, which was consistent with the work of other authors[18,20]. Since γ-IFN can upregulate Fas expression and make target cells susceptible to Fas-mediated apoptosis[10,12], such suppression on haematopoiesis by the serum is probably due to the induction of apoptosis in the progenitor cells. To test this hypothesis and also to investigate the involvement of apoptosis in the pathogenesis of aplastic anemia, we measured the percentage of the apoptotic cells and the Fas-R in CD34$^+$ cells from the bone marrow of normal donors after incubation with serum from normal controls and patients with aplastic anemia, analyzing the possible role of γ-IFN in the serum from the AA patients.

MATERIALS AND METHODS

Serum sampling

Blood samples for this study were obtained from 10 normal controls and 23 AA patients (17 males and 6 females, ranging from 14 to 72 years old)

Table 1. Clinical and laboratory findings of patients with aplastic anemia

Patient	Age/sex	Peripheral blood				Severity
		HB (g/dl)	Leukocytes ($\times 10^9$/liter)	Neutrophils ($\times 10^9$/liter)	Platelets ($\times 10^9$/liter)	
1	35/M	5.6	0.6	0.3	19.0	SAA
2	14/F	4.2	0.8	0.4	14.0	SAA
3	66/F	5.6	0.6	0.3	15.0	SAA
4	58/M	8.6	1.1	0.3	5.0	SAA
5	56/M	7.5	0.7	0.3	12.0	SAA
6	15/M	7.2	0.8	0.2	20.0	SAA
7	72/M	4.5	1.0	0.4	19.0	SAA
8	36/M	7.3	0.7	0.2	7.0	SAA
9	28/M	4.5	1.3	0.4	8.0	SAA
10	38/M	7.2	2.3	0.5	19.0	SAA
11	20/F	8.2	2.5	1.6	23.0	NSAA
12	58/M	9.3	2.8	1.4	32.0	NSAA
13	44/M	7.5	1.0	0.5	75.0	NSAA
14	26/M	8.2	2.0	1.4	82.0	NSAA
15	59/M	7.2	3.3	1.4	48.0	NSAA
16	29/F	5.6	2.6	0.8	22.0	NSAA
17	52/M	7.8	3.1	1.3	32.0	NSAA
18	36/M	8.1	2.3	0.9	10.0	NSAA
19	41/M	7.0	2.3	0.7	25.0	NSAA
20	41/M	6.3	3.1	1.4	45.0	NSAA
21	18/F	5.4	2.6	1.8	52.0	NSAA
22	49/M	4.6	3.2	1.6	38.0	NSAA
23	38/F	5.4	3.2	1.8	28.0	NSAA

after informed consent obtained. The diagnosis of AA was established by peripheral blood pancytopenia associated with a depletion of all the hemopoietic cell lines in the marrow with replacement by fat cells, an absence of abnormal cells or dysplastic features, no increase in marrow reticulin and negative Ham's test. The severity of AA was classified according to the criteria of Bacigalupo et al[1]. Ten patients had SAA, and 13 NSAA. Patients 2, 9, and 12 had suffered from hepatitis (B or non-A non-B) in their past history and had had a pancytopenia within 8 months. The disease of patients 4 and 10 was considered to be related to medical drugs used in treatment. No possible causation was suspected for the other AA patients. Prior to the present study, patients 1, 2, 3, 6, 13, 15, 16 and 21 had received blood transfusions. Other patients had received no specific treatment. Clinical features are shown in Table 1. All these blood samples were centrifuged after being allowed to clot at room temperature for one hour. The serum recovered was divided into several aliquots and stored at –20°C until use.

Preparation of CD34$^+$ cells

CD34$^+$ cells were isolated from the bone marrow of normal volunteers in the following way after obtaining informed consent: bone marrow was aspirated from the posterior iliac crest into syringes containing preservation free heparin. Mononuclear cells were obtained by density gradient centrifugation using lymphoprep (Nycomed Pharma, AS. Oslo, Norway). CD34$^+$ cells were then collected by incubation with commercial magnetic microbeads coated with MoAb to CD34 (Miltenyi Biotec, Inc., Auburn, CA), according to the manufacturer's instructions. More than 95% of these isolated cells expressed CD34$^+$ when analyzed by flow cytometry.

Serum-induced apoptosis assay

Isolated CD34$^+$ cells were resuspended in RPMI 1640 medium with 5% fetal calf serum to a final concentration of 5×10^3 cells /ml. Aliquots of 1ml were incubated in the presence of 10% (V/V) sera from normal controls or AA patients at 37°C in 5% CO_2 for 24hr and 48hr, respectively. Cultures without human serum were used to measure spontaneous apoptosis. Apoptotic cells were quantified by flow cytometry after AnnexinV-FITC labeling (R&D Systems) as described by King et al[7]. The value of serum-induced apoptotic cells was defined as the percentage of apoptotic cells after incubation with serum minus the percentage of spontaneous apoptosis (Fig. 1).

Detection of Fas-R induced by the serum

Fas-R on CD34$^+$ cells was immunophenotyped using flow cytometry. Briefly, isolated CD34$^+$ cells were collected after culture with the human serum as mentioned above and then incubated with a saturating dose of anti-Fas monoclonal antibody (MoAb) (MBL, Nagoya, Japan) for 30 min. After 3 washes, PE-conjugated goat anti-mouse IgG

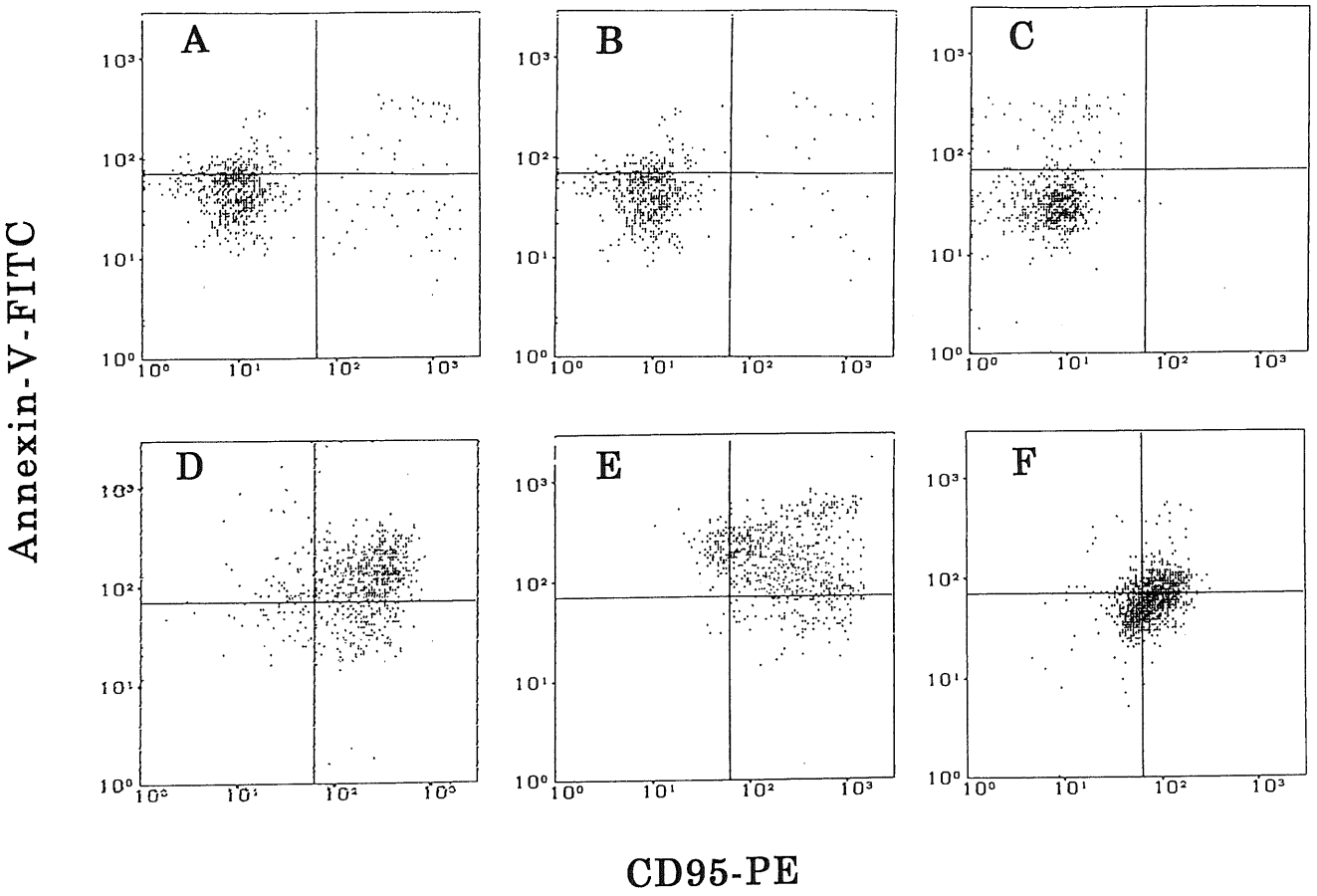

Fig. 1. Flow cytometric analysis of apoptosis and Fas-R expression in $CD34^+$ cells from bone marrow of normal donors after 24hr incuvation under different experimental conditions
(A) $CD34^+$ cells plus medium (spontanous apoptosis)
(B) $CD34^+$ cells plus medium and anti-γ-IFN MoAb
(C) $CD34^+$ cells plus serum from a normal control
(D) $CD34^+$ cells plus serum from patient 4
(E) $CD34^+$ cells plus serum from patient 8
(F) $CD34^+$ cells plus serum from patient 8 after preincubation with anti-γ-IFN MoAb

$F(ab')_2$ fragment (Ortho Diagnostic Systems Inc., Raritan., N.J.) was used as the second layer. Finally, after washing with PBS, the stained cells were analyzed by an Epics Elics flow cytometer.

Serum neutralization test

The experiment to block the apoptosis and the expression of Fas-R induced by the sera of AA patients with anti-γ-IFN neutralizing MoAb (Genzyme, Cambridge, MA) was performed according to the manufacturer's instructions. After having been neutralized with anti-γ-IFN MoAb, serum samples were then added to the culture as described above.

Statistical analysis

Data were analyzed with student's t-test for paired and unpaired samples and with linear regression analysis. P value<0.05 was considered to indicate a significant difference.

RESULTS

The percentage of apoptosis in $CD34^+$ progenitors induced by human serum was plotted and is shown in Fig. 2. The number of apoptotic cells in $CD34^+$ progenitors significantly increased after both 24 and 48hr incubation with serum from SAA patients compared with those incubated with serum from the normal controls ($p<0.001$, $p<0.001$) and NSAA ($p<0.001$, $p<0.001$). The percent apoptosis of $CD34^+$ cells was slightly higher after 24hr incubation with serum from the NSAA patients than that of cells incubated with serum from the normal controls ($p<0.05$). No significant difference was found in the percent apoptosis of $CD34^+$ cells between 24 and 48hr incubation with the same serum ($p>0.5$). In addition, the number of leukocytes in the AA patients was inversely related to the increased apoptosis of $CD34^+$ cells induced by the serum from AA patients ($r=-0.528$, $p<0.01$) (Fig. 3). However, no significant correlation was found between the number of platelets and the serum-induced apoptosis of $CD34^+$ cells

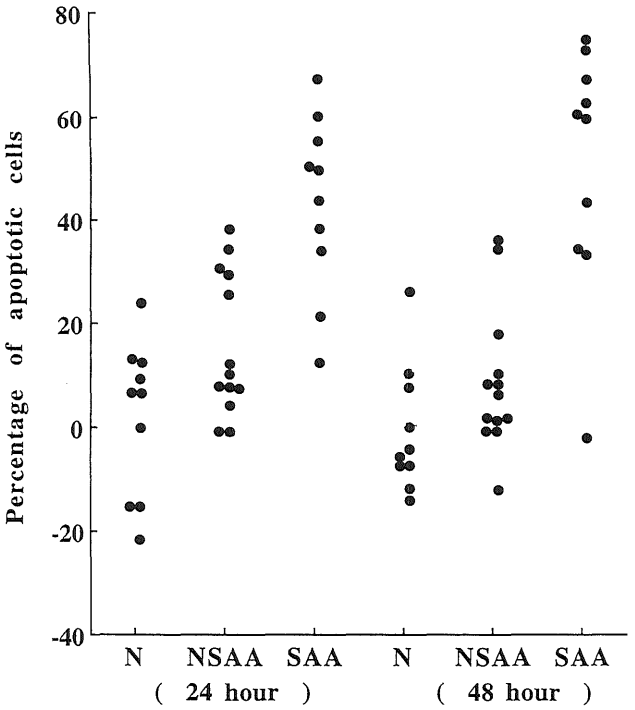

Fig. 2. Percentage of sera-induced apoptosis in CD34⁺ cells from bone marrow of normal donors after 24hr and 48hr incubation with serum of normal controls (n=10), patients with NSAA (n=13) and patients with SAA (n=10). The value of serum-induced apoptotic cells was defined as the percentage of apoptotic cells after incubation with serum minus the percentage of spontaneous apoptosis.

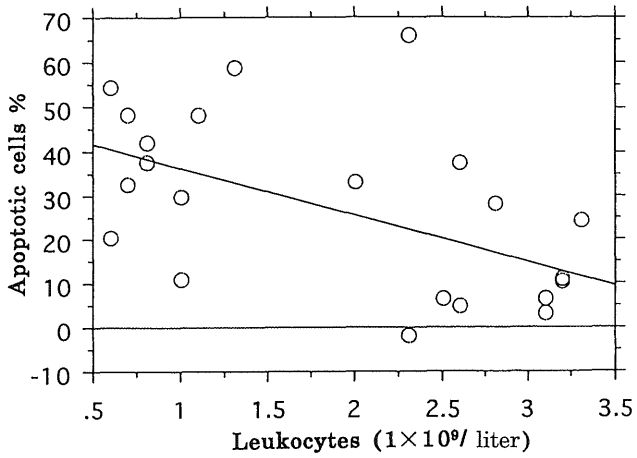

Fig. 3. Correlation between increased apoptosis in CD34⁺ cells induced by serum from AA patients (n=23) and the severity of leukocytopenia in the same patients.

(r=–0.277, p=0.203) (Fig. 4).

To determine the pathway of apoptosis induced by the serum from AA patients, the expression of the Fas-R on the CD34⁺ cells was measured after incubation with serum from the normal controls and AA patients. Flow cytometric analysis revealed that Fas-R was overexpressed after 24hr

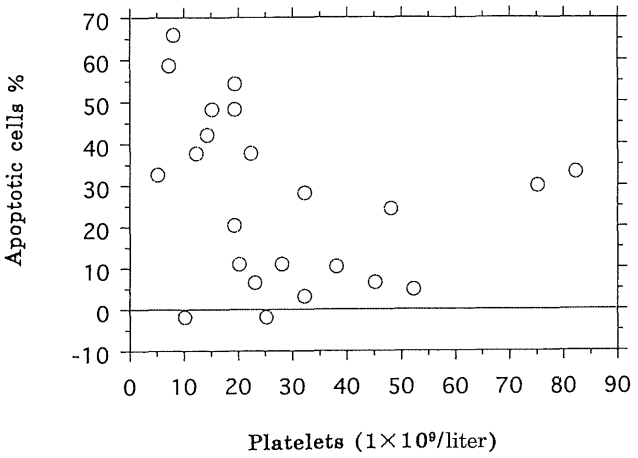

Fig. 4. Relationship between the percentage of apoptosis in CD34⁺ cells induced by serum from AA patients (n=23) and the number of platelets in the same patients.

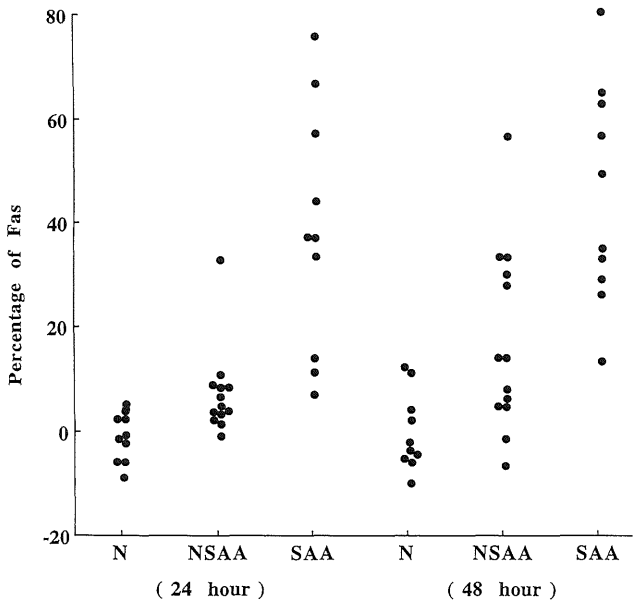

Fig. 5. Percentage of serum-induced Fas expression in CD34⁺ cells from bone marrow of normal donors after 24hr and 48hr incubation with serum from normal controls (n=10), patients with NSAA (n=13) and patients with SAA (n=10)

incubation with serum from the SAA patients compared with incubation with serum from the normal controls and NSAA patients ($p<0.001$, $p<0.001$). Fas-R expression of normal CD34⁺ cells was also increased by exposure to serum from NSAA patients for 24hr compared with that of cells exposed to serum from the normal controls ($p<0.05$). Moreover, the same results were obtained with the prolonged incubation time of 48hr (Fig. 5).

The correlation coefficient between the number of apoptotic cells induced by serum from AA patients and Fas-R expression on CD34⁺ cells induced by the same serum was 0.617 ($p<0.001$). In other

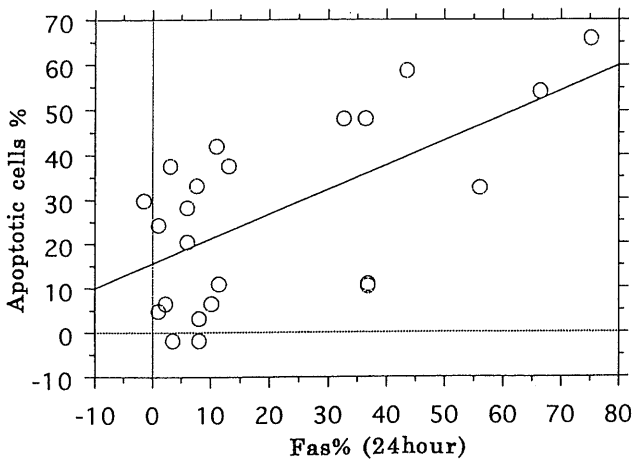

Fig. 6. Correlation between apoptotic cells induced by serum from AA patients (n=23) and Fas-R expression on CD34⁺ cells induced by the same serum samples.

words, the number of apoptotic cells correlated with the expression of Fas-R on CD34⁺ cells (Fig. 6). These findings suggested that the serum of AA patients induced apoptosis of CD34⁺ cells through the Fas signaling pathway.

To further explore the mechanism of the increased apoptosis induced by serum from the AA patients, a neutralization test was performed using serum treated with anti-γ-IFN MoAb. As shown in Table 2 and Fig. 1, the effect on the induction of apoptosis and the Fas-R expression of the CD34⁺ cells caused by serum from patients 2 and 9 was almost completely blocked after incubation with the MoAb. Both of them were post hepatitis. The same effect of the serum from patients 1, 3, 4, 5, 6, 7 and 8 was partly blocked after incubation with the MoAb. However, no significant difference in the number of apoptotic cells and Fas-R expression on CD34⁺ cells was observed before and after neutralizing the serum from patient 10 with the MoAb. To exclude nonspecific effects of the anti-γ-IFN MoAb on the CD34⁺ cells, the effect of the MoAb on the CD34⁺ cells was examined for apoptosis and Fas-R expression in the presence or absence of the control serum samples. As shown in Fig. 1 and Table 2, the MoAb did not show any significant effect on normal progenitor cells.

DISCUSSION

Homeostasis in humans is controlled not only by cell proliferation and differentiation, but also by cell death. Apoptosis is a morphologically defined death process, which plays an important role in physiological cell death and is also important in pathological cell destruction in AA patients[2,3,5,11]. There are many possible mechanisms underlying the hematopoietic stem cell deficiency present in aplastic bone marrow. An intrinsic abnormality in the stem cell itself could render it more sensitive to apoptosis-inducing stimuli; alternatively, the activity of the bone marrow microenvironment in AA patients may alter the balance of apoptosis-promoting and -suppressing regulating molecules. In the present study, we examined the effect of AA serum on normal CD34⁺ progenitors and demonstrated that the serum could induce CD34⁺ cells apoptosis, correlating with the severity of the leukocytopenia. Although no correlation was found between serum-induced apoptosis and the number of platelets in the AA patients, the serum from patients with SAA showed a stronger effect than that from patients with NSAA, suggesting the presence of some aberrant component in the serum from the AA patients. This could have induced the CD34⁺ progenitor apoptosis and might be a part of the pathogenesis of AA.

There is a possibility that the serum from the AA patients did not induce CD34⁺ cell apoptosis directly, but stimulated the monocytes and lymphocytes in the target cells to produce the cytokines which induced the apoptosis. Our experiment using purified CD34⁺ cells as the target cells ruled out the possibility mentioned above.

Fas-R, a transmembrane glycoprotein of 43- to 48-KD, is a member of the TNF receptor superfamily and its triggering by the natural ligand or

Table 2. Effect of anti-γ-IFN MoAb on apoptosis and Fas-R expression of CD34⁺ cells induced by serum from SAA patients

Source of Serum	Apoptotic cell		Percentage of Fas-R	
	Before neutralization	After neutralization	Before neutralization	After neutralization
Patient 1	20.4	11.5	5.8	3.9
Patient 2	42.5	3.3	10.7	–3.1
Patient 3	48.5	14.6	32.4	17.2
Patient 4	33.0	24.9	36.1	25.7
Patient 5	37.6	10.9	43.0	15.3
Patient 6	48.6	23.1	74.8	–0.1
Patient 7	59.0	30.3	66.1	45.7
Patient 8	66.0	23.5	56.0	45.1
Patient 9	54.3	–6.8	12.9	2.7
Patient 10	11.9	15.6	36.6	35.8

by anti-Fas antibody induced apoptosis[5,17,19]. Fas-mediated killing has been suggested as a major effector mechanism by which T cells eliminate their targets[4,6,16]. Although $CD34^+$ cells from normal bone marrow showed low levels of Fas-R expression on the cell surface, stimulation by γ-IFN markedly increased Fas-R expression on the $CD34^+$ cells[10,12]. In the present study, the Fas-R on the $CD34^+$ cells from normal bone marrow was overexpressed after incubation with serum from AA patients, which correlated with the increased apoptosis induced by the same serum. Therefore, the increased apoptosis of the $CD34^+$ progenitors induced by the serum from the AA patients seemed to have occurred through the Fas signaling pathway. In addition to the Fas-R/Fas-ligand pathway, perforin/granzyme is a second exclusive mechanism by which target cells can be eliminated[4,6]. As our study only examined Fas-R expression, we cannot exclude the possibility of the involvement of perforin/granzyme in the pathophysiology of BM failure.

A relationship has been reported between aplastic anemia and γ-IFN, an immunoregulatory cytokine produced by active T cells and natural killer cells in response to viral infection or other stimuli. Some studies have demonstrated aberrant γ-IFN gene expression locally in aplastic bone marrow[13,14]. Additional investigations found that the serum γ-IFN level was higher in AA patients and anti-γ-IFN antibody neutralized the suppressive effect of the serum from AA patients[8,18,21]. Therefore, γ-IFN has been proposed as an inhibitor in AA patients. Since γ-IFN was confirmed to induce Fas-R expression on progenitor cells in low doses, we performed a neutralization test treating serum with anti-γ-IFN MoAb to determine whether the aberrant component inducing the apoptosis of $CD34^+$ cells was γ-IFN. The induction of apoptosis and Fas-R expression on the $CD34^+$ cells was blocked completely (patients2, 9) or partly (patients 1, 3, 4, 5, 6, 7, and 8) by preincubating the serum from the SAA patients with anti-γ-IFN neutralizing MoAb, suggesting that γ-IFN is a major factor in serum that induces apoptosis of the $CD34^+$ progenitors. This observation thus suggests a mechanism responsible for at least part of the inhibitory effect of serum from AA patients on human progenitor cells that we described previously[8].

Serum from patients 2 and 9, who had a previous history of hepatitis, showed a complete blocking effect on the $CD34^+$ progenitors by anti-γ-IFN MoAb. It is possible that hepatitis virus infection activated T cells abnormally and that these T lymphocytes produced γ-IFN, resulting in peripheral blood pancytopenia and bone marrow hypocellularity by accelerating apoptosis of the progenitor cells. However, no pathogen causing aplastic anemia was found in other patients whose serum was partly neutralized by the MoAb. It seems that some form of immune disorder or latent virus infection precedes some cases of idiopathic aplastic anemia and induces γ-IFN production. In fact, patient 4 had a higher lymphocyte count with $CD8^+$ than the controls and responded well to anti-lymphocyte globulin. The result supports the possibility mentioned above.

Anti-γ-IFN MoAb did not block the effect of the serum from the SAA patients on apoptosis and Fas-R expression of the $CD34^+$ cells completely. This result suggested that γ-IFN was not the only factor present in the serum of AA patients that induced the apoptosis of the $CD34^+$ cells. Some reports have indicated that TNF-α, well known as a negative hematopoietic regulator, induces a strong expression of functional Fas on hematopoietic progenitor cells[10,12]. Further studies will be necessary to identify the other factors involved in the apoptosis process in AA patients.

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REFERENCES

1. **Bacigalupo, A., Howes, J., Gluckman, E., Nissen, C., Marsh, J., Vanlint, M.T., Congiu, M., de Planque, M.M., Ernst, P., Mccann, S., Ragavachar, A., Frickhofen, N., Wursch, A., Marmont, A.M. and Gordon-Smith, E.C.** 1988. Bone marrow transplantation (BMT) versus immunosuppression for the treatment of severe aplastic anemia(SAA): A report of the EBMT SAA working party. Br. J. Haematol. **70:** 177–182.
2. **Callera, F. and Falcao, R. P.** 1997. Increased apoptosis cells in bone marrow biopsies from patients with aplastic anemia. Br. J. Haematol. **98:** 18–20.
3. **Callera, F., Garcia, A.B. and Falcao, R.P.** 1998. Fas-mediated apoptosis with normal expression of bcl-2 and p53 in lymphocytes from aplastic anemia. Br. J. Haematol. **100:** 693–703.
4. **Hanabuchi, S., Koyanagi, M., Kawasaki, A., Shinohara, N., Matsuzawa, A., Nishimura, Y., Kobayashi, Y., Yonehara,Y., Yagita, H. and Okumura, K.** 1994. Fas and its ligand in a general mechanism of T-cell-mediated cytotoxicity. Pro. Natl. Acad. Sci. **91:** 4930–4934.
5. **Itoh, N., Yonehara, S., Ishii, A., Yonehara, M., Mizushima, S.L., Sameshima, M., Hase, A., Seto, Y. and Nagata, S.** 1991. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell **66:** 233–243.
6. **Kagi, D., Vignaux, F., Ledermenn, B., Burki, K., Depraetere, V., Nagata, S., Hengartner, H. and**

Golstein, P. 1994. Fas and perforin pathways are major mechanisms of T-cell mediated cytotoxicity. Science **265:** 528–529.

7. **King, D., Pringle, J.H., Hutchinson, M. and Cohen, G.M.** 1998. Processing/activation of caspases,-3 and -7 and-8 but not caspase -2 in the induction of apoptosis in B-chronic lymphocytic leukemia cells. Leukemia **12:** 1553–1560.
8. **Liu, H., Ding, R.S., Jiang, S.H., Yang, J.Y., Chen, H.Q., Lu, D.Y., Zi, L.Y. and Wu, F.Y.** 1996. Suppression of haematopoiesis by sera from patients with aplastic anemia. Tokushima J. Exp. Med. **43:** 107–111.
9. **Liu, H., Ozaki, K., Matsuzaki, Y., Abe, M., Kosaka, M. and Saito, S.** 1995. Suppression of haematopoiesis by IgG autoantibodies from patients with systemic lupus erythematosis (SLE). Clin. Exp. Immunol. **100:** 480–485.
10. **Maciejewski, J.P., Selleri, C., Anderson, S. and Young, N.S.** 1995. Fas antigen expression on $CD34^+$ human marrow cells is induced by interferon-γ and tumor necrosis factor-α and potentiates cytokine-mediated hematopoietic suppression in vitro. Blood **85:** 3183–3190.
11. **Maciejeuski, J.P., Seller, C., Sato, T., Anderson, S. and Young, N.S.** 1995. Increased expression of Fas antigen on bone marrow $CD34^+$ cells of patients with aplastic anemia. Br. J. Haematol. **91:** 245–252.
12. **Nagafuji, K., Shibuya, T., Harada, M., Mizuno, S., Takenaka, K., Miyamoto, T., Kamura, T., Gondo, H. and Niho, Y.** 1995. Functional expression of Fas antigen (CD95) on hematopoietic progenitor cells. Blood **83:** 883–889.
13. **Nakao, S., Yamaguchi, M., Shiobara, S., Yokoi, T., Miyawaki, T., Taniguchi, T. and Matsuda, T.** 1992. Interferon gamma gene expression in unstimulated bone marrow mononuclear cells predicts a response to cyclosporine therapy in aplastic anemia. Blood **79:** 2532–2535.
14. **Nistico, A. and Young, N.S.** 1994. Gamma-interferon gene expression in the bone marrow of patients with acquired aplastic anemia. Anal. Inter. Med. **120:** 463–469.
15. **Philpott, N.J., Scopes, J., Marsch, J.C.W. and Gibson, F.M.** 1995. Increased apoptosis in aplastic anemia bone marrow progenitor cells:possible pathophysiologic significance. Exp. Haematol. **23:** 1642–1648.
16. **Rouvier, E., Luciani, M.F. and Golstein, P.** 1995 Fas involvement in Ca^{2+}-independent T-cell-mediated cytotoxicity. J. Exp. Med. **177:** 195–200.
17. **Suda, T., Takahashi, T., Golstein, P. and Nagata, S.** 1993. Molecular cloning and expression of tumor necrosis family. Cell **75:** 1169–1178.
18. **Tohda, S., Suzuki, T., Nagata, K., Nara, N. and Aoki, N.** 1990. Haematopoietic suppressing activity of gamma-interferon in serum and bone marrow of aplastic anemia patients. Acta. Haematol. Jap. **53:** 14–20.
19. **Watanabe-Fukunaga, R., Brannan, C.I., Itoh, N., Yonehara, S., Copeland, N.G., Jenkins, N.A. and Nagata, S.** 1992. The cDNA structure,expression and chromosomal assignment of the mouse Fas antigen. J. Immunol. **148:** 1274–1279.
20. **Zoumbos, N.C., Djeu, J.Y. and Young, N.S.** 1984. Interferon is the suppressor of hematopoiesis generated by stimulated lymphocytes in vitro. J. Immunol. **133:** 769–774.
21. **Zoumbos, N.C., Gascon, P., Djeu, J.Y. and Young, N.S.** 1985. Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. Pro. Natl. Acad. Sci. **82:** 188–192.