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—Original Article—

Effects of recombinant interleukin 2 on immunological effector cells of the peripheral blood in patients with HBe antigen-positive chronic hepatitis

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Summary: Changes in peripheral blood immunological effector cells after systemic administration of recombinant interleukin 2 (rIL2) were studied in 14 patients with HBe antigen-positive chronic hepatitis. The patients were intravenously injected with rIL2 for 4 weeks, and the experiments were performed using mononuclear cells isolated from the peripheral blood during and after rIL2 administration. No significant changes in OKT4-positive cell population, OKT8-positive cell population, OKT4/OKT8 ratio and Leu7-positive cell population were detected during and after intravenous injection of rIL2. However, the natural killer (NK) cell and lymphokine-activated killer (LAK) cell activities significantly increased in the fourth week of rIL2 administration, while the K cell activity in the antibody-dependent cell-mediated cytotoxicity reaction, antibody response, lectin-induced lymphocyte transformation and IL1 production of LPS-stimulated monocytes were not affected. These results suggest that IL2 increases the NK cell and LAK cell activities *in vivo*. *Gastroenterol Jpn 1988;23:147–152*

Key Words: *LAK cell, NK cell, Recombinant interleukin 2*

Introduction

Interleukin 2 (IL2), a lymphokine produced by activated T cells, is mainly involved in the proliferation of T cells, but it also directly or indirectly affects other lymphocytes and plays an important role in their proliferation and differentiation. Cells that respond to IL2 have IL2 receptors which are activated by stimulation with antigen through antigen-presenting cells and by the production of IL1[1,2]. These cells that respond to IL2 stimulation include helper T cells, cytotoxic T cells and natural killer (NK) cells, and IL2 is also known to increase the production of γ-interferon. Recently, the clinical effects of lymphokine-activated killer (LAK) cells activated by direct IL2 stimulation have been increasingly noted[3-5]. Remarkable progress has been made in the clinical application of IL2 since the development of recombinant IL2 (rIL2) produced by genetic manipulation. It has been used in the treatment of acquired immune deficiency syndrome (AIDS) and cancer, and its effectiveness in adoptive immunotherapy has been indicated[6-9]. It is also known to be effective in the elimination of viruses in viral infections and in the immunological removal of virus-infected cells[10]. We have applied rIL2 in the treatment of patients with HBe antigen-positive chronic hepatitis and found that it decreased the serum activity of DNA polymerase (DNA-P) produced by the virus[11]. In this

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Table 1 Patient profile.

Case	Age	Sex	GOT(IU)	HBeAg	HBeAb	DNA-P (cpm)	Biopsy	Dose of rIL2(IU)xdays
1	37	M	70	(+)	(−)	0	CAH	250 × 7
								500 × 7
2	52	F	120	(+)	(−)	0	CIH	500 × 14
3	31	M	147	(+)	(−)	78	CIH	500 × 7
4	45	M	161	(+)	(−)	878	CAH	250 × 7
5	22	M	160	(+)	(−)	1764	CIH	500 × 7
								750 × 7
6	41	F	70	(+)	(−)	314	CIH	500 × 21
								750 × 4
								1000 × 3
7	37	M	79	(+)	(−)	120	CAH	250 × 1
								500 × 27
8	37	M	104	(+)	(−)	3416	CIH	250 × 1
								500 × 27
9	39	M	28	(+)	(−)	2244	CIH	250 × 1
								500 × 2
								750 × 25
10	36	M	215	(+)	(−)	238	CAH	250 × 1
								750 × 27
11	28	F	337	(+)	(−)	2828	CAH	500 × 28
12	29	M	84	(+)	(−)	352	CAH	250 × 28
13	23	M	107	(+)	(−)	2310	CIH	500 × 4
								750 × 4
								1000 × 3
								750 × 17
14	31	M	142	(+)	(−)	0	CIH	250 × 1
								500 × 1
								750 × 5
								1000 × 21

study, we administered rIL2 to 14 patients with HBe antigen-positive chronic hepatitis and evaluated the changes in various immunological parameters during and after rIL2 administration.

Materials and Methods

1. Materials

Fourteen patients (11 males and 3 females, 22 to 45 years old) with HBe antigen-positive chronic hepatitis were selected for the study. The patients had not been treated with interferon, Ara-A or corticosteroids during the 6 months before rIL2 administration.

2. Administration of rIL2

rIL2 produced from human peripheral blood lymphocytes by recombinant technology was provided by Takeda Chemical Industries Company, Osaka, Japan. This purified protein was homogenous on sodium dodecyl sulfate polyacrylamide gel. The material was more than 95% pure, and specific activity was 3.5×10^4 units/mg. One unit of IL2 was defined as the amount of activity in the conditioned medium of human peripheral blood lymhocytes (5×10^6 cells/ml) cultured in the presence of TPA (15 ng/ml, 12-o-tetradecanoyl-phorbol-13-acetate) and Concanavalin A (20 μg/ml) for 48 yours[12]. It corresponded to 1.2×10^7 units/mg calculated on the basis of the Biological Response Modifiers Program reference reagent human IL2 prepared from the Jurkat cell line[13]. Various *in vitro* biological activities of rIL2 were comparable to those of natural IL2 on the basis of protein weight[14]. This rIL2 was dissolved in 500 ml of 5% glucose or saline solution, and 250

to 1000 units/day was intravenously infused for 1 to 4 weeks. Peripheral blood was obtained from the patients before, during and after rIL2 administration and used for the experiments. Patient profile and dose of rIL2 are indicated in **Table 1**. None of the patients had any complications from the administration of rIL2.

3. Immunological studies

1) Analysis of lymphocyte subsets

Peripheral blood lymphocytes were obtained from the patients and treated with FITC-conjugated monoclonal antibody (OKT4, OKT8 and Leu7). OKT4, OKT8 and Leu7-positive cells were separated by laser flow cytometry (Spectrum III — Ortho Co.,), and the percentage of total lymphocytes was determined. The OKT4/OKT8 ratio was also calculated.

2) Measurement of NK cell activity

Heparinized peripheral blood was obtained from the patients, and the mononuclear cells were separated by Ficoll-Conray density centrifugation. The cells were washed with RPMI 1640 medium containing 10% fetal calf serum and suspended in the same medium to produce a cell suspension of 1×10^6 cells/ml. ^{51}Cr-labeled K-562 cells were used as target cells and added to the cell suspension at an E/T ratio of 20:1. This was cultured for 3.5 hours, and the culture supernatant was separated by centrifugation. The radioactivity of the supernatant was measured with a γ-counter to determine the NK cell activity. Percent cytotoxicity was calculated as follows:

$$\frac{\text{experimental } ^{51}\text{Cr separated} - \text{natural } ^{51}\text{Cr separated}}{\text{maximum } ^{51}\text{Cr separated} - \text{natural } ^{51}\text{Cr separated}} \times 100$$

3) Measurement of LAK cell activity

Peripheral blood mononuclear cells (1×10^6 cells/ml) were obtained as described before and cultured with rIL2 (20 ng/ml) at 37°C for 72 hours. Daudi cells were used as target cells. The cells were washed with RPMI 1640 medium containing 10% fetal calf serum and added to the mononuclear cells at a ratio of 50:1. This was cultured for 24 hours, and the LAK cell activity was estimated by the trypan blue dye exclusion test determining target cell viability.

4) Measurement of K cell activity in antibody-dependent cell-mediated cytotoxicity (ADCC) reaction

Peripheral blood mononuclear cells (1×10^6 cells/ml) were obtained as described before, and the K cell activity was measured using chicken red blood cells (CRBC) as target cells. Anti-CRBC was added to ^{51}Cr-labeled CRBC, and this was cultured for 30 min. These cells were added to the mononuclear cells at a ratio of 10:1, and this was cultured for 20 hours. The culture supernatant was separated by centrifugation, and the radioactivity of the separated ^{51}Cr was measured with a γ-counter[15].

5) Lymphocyte transformation test

Peripheral blood mononuclear cells obtained as described above were washed with RPMI 1640 medium and suspended in RPMI 1640 medium containing 10% fetal calf serum to make a cell suspension of 5×10^5 cells/ml. Ten μg/ml of phytohemagglutinin (PHA; Difco Co., Detroit) or Concanavalin A (Con A; Sigma Co., St. Louis, MO) was added to the cell suspension, and this was incubated for 48 or 72 hours. ^{3}H-thymidine (1 μCi/ml, specific activity: 5 Ci/mmol) was added, and incubation was continued for another 24 hours. The cells were filtered through a millipore filter, and the radioactivity incorporated into the acid-insoluble fraction was measured with a liquid scintillation counter.

6) Measurement of antibody production

Pokeweed mitogen (PWM; Difco Co., 10 μg/ml) was added to the peripheral blood mononuclear cell suspension (1×10^6 cells/ml) prepared as described above, and this was incubated for 72 hours. Polyclonal antibody response induced by PWM was estimated by measuring antibody-forming cells produced against trinitrophenylated sheep red blood cells (TNP-SRBC) using hemolytic plaque assay according to the method of Jerne et al[16].

7) Measurement of IL1 production

Lipopolysaccharide (LPS; Difco Co., 50 μg/ml) was added to the peripheral blood mononuclear cell suspension (1×10^6 cells/ml) prepared as

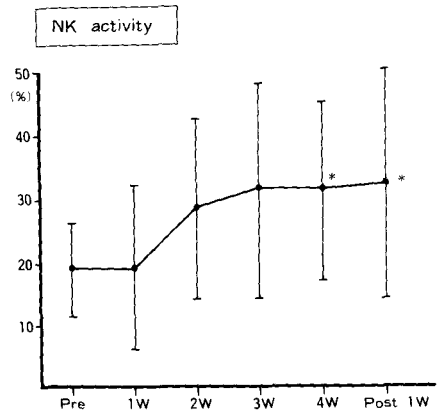

Fig. 1 Changes in HK cell activity in peripheral blood from patients with HBe antigen-positive chronic hepatitis during and after rIL2 administration.
All values are means ± SD.
Significant difference *p<0.05.
Fourteen patients were followed each week.

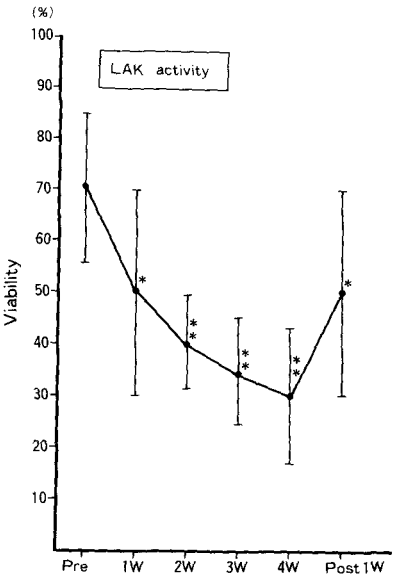

Fig. 2 Changes in LAK cell activity in peripheral blood from patients with HBe antigen-positive chronic hepatitis during and after rIL2 administration.
All values are means ± SD.
Significant difference *p<0.05, **p<0.01.
Fourteen patients were followed each week.

described before, and this was incubated for 48 hours. The culture supernatant separated by centrifugation and PHA (2 μg/ml) were added to the thymocyte suspension prepared from C3H/HeJ mice. After 48 hours of incubation, ^{3}H-thymidine (1 μCi/ml) was added, and this was incubated for another 24 hours. The radioactivity incorporated into the acid-insoluble fraction was measured with a liquid scintillation counter to determine IL1 activity.

8) Statistical analysis

All results are indicated as means ±SD. Significant differences were calculated by analysis of the t-test.

Results

1. Changes in lymphocyte subsets

Mean percentage of OKT4-positive cells, the marker for helper/inducer T cells, in the lymphocytes did not significantly change with the administration of rIL2. Mean values for OKT8-positive cells, the marker for suppressor/cytotoxic T cells, also showed no significant change. As a result, the OKT4/OKT8 ratio also did not significantly change. Similarly, Leu7, the marker for NK cells, did not show any significant change.

2. Changes in NK cell activity

As shown in **Figure 1**, the NK cell activity of the peripheral blood tended to increase from the second or third week of rIL2 administration, and a significant increase was seen at the fourth week, or one week after rIL2 administration ($p<0.05$).

3. Changes in LAK cell activity

As shown in **Figure 2**, the LAK cell activity of the peripheral blood increased from the second or third week of rIL2 administration, and a significant increase was seen at the fourth week ($p<0.01$).

4. K cell activity in ADCC reaction

The K cell activity of the peripheral blood mononuclear cells in the ADCC reaction hardly changed with administration of rIL2.

5. Response to PHA and Con A stimulation

The lymphocyte transformation test indicated that the response of the peripheral blood lymphocytes to PHA and Con A stimulation did not significantly change with the administration of rIL2. In addition, when PHA and Con A

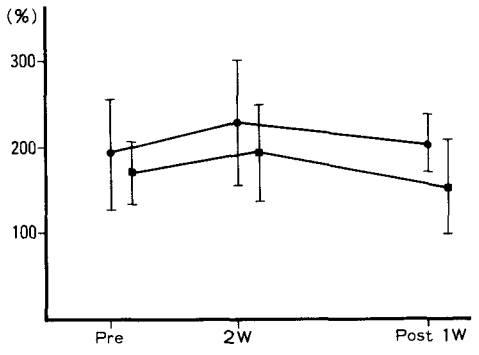

Fig. 3 Changes in antibody response of peripheral blood mononuclear cells from patients with HBe antigen-positive chronic hepatitis during and after rIL2 administration.
All values are means ± SD.
●—● IgM ■—■ IgG
Fourteen patients were followed each week.

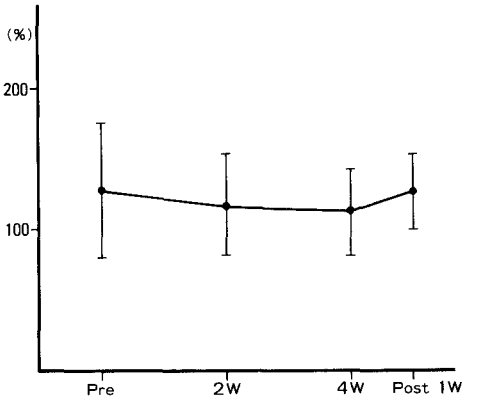

Fig. 4 Changes in IL1 production of peripheral blood monocytes from patients with HBe antigen-positive chronic hepatitis during and after rIL2 administration.
All values are means ± SD.
Fourteen patients were followed each week.

were not added, the incorporation of ^{3}H-thymidine was not different from that of the healthy controls.

6. Antibody production

There was no change in the number of anti-TNP-SRBC plaque-forming cells, indicating that antibody production of the peripheral blood mononuclear cells did not change with the administration of rIL2 (**Fig. 3**).

7. IL1 production

No significant change in IL1 production of the LPS-stimulated monocytes was observed with the administration of rIL2 (**Fig. 4**).

However, none of the parameters studied including lymphocyte subsets, NK cell activity, LAK cell activity, K cell activity, lymphocyte transformation, antibody production and Il1 production were affected by the dose of rIL2.

Discussion

IL2 has been shown to have various immuno-enhancing effects *in vitro*, and *in vivo*, it has been reported that IL2 increased the immune response to hetroantigens and alloantigens in nude mice[17], and that it restored the decrease in the immune response to alloantigens induced by Cytoxan[18]. Other reports have shown that when purified IL2 was administered to normal mice, the NK cell activity increased and the immune response to alloantigens was enhanced[18]. Since *in vivo* experiments using IL2 require comparatively large amounts of purified IL2, it has been difficult to administer it in patients and study its clinical effects. However, with the development of genetic engineering, rIL2 has become widely available, and is being applied to enhance the immune response in cases involving various immun-deficient conditions such as AIDS or cancer. Since IL2 increases γ-interferon production, it has also been used in the treatment of chronic hepatitis B with favorable results, as indicated by the decrease in serum DNA-P activity[11].

In this report, we studied 14 patients with HBe antigen-positive chronic hepatitis, and found that mean levels of helper/inducer T cells (OKT4-positive), suppressor/cytotoxic T cells (OKT8-positive) and NK cells (Leu7-positive) did not significantly change with the administration of rIL2, only showing slight changes in some patients. The K cell activity of peripheral blood mononuclear cells in the ADCC reaction, response of T cells to mitogen stimulation, antibody production induced by PWM stimulation and IL1 production due to LPS stimulation were also unaffected by the administration of rIL2. These results may be due to the short half-

life period of rIL2 in venous blood, so that the method of administration must be improved. Furthermore, it is suggested that because IL2 activates the lymphocytes which respond to antigen stimulation or the cells with IL2 receptors, it could not exert significant effects by this experimental method.

In contrast to the lack of these changes, the NK cell and LAK cell activities of the peripheral blood significantly increased with the administration of rIL2. Chang et al.[19] have also reported that when human rIL2 was administered to mice, the NK cell activity was enhanced, indicating that the systemic administration of rIL2 affects the NK cells. They have reported that the half-life period of intravenously-injected rIL2 was 1.6 ± 0.3 min, which was extended when injected peritoneally or percutaneously, and that when IL2 was mixed with 15% gelatin, the period was further extended. These results indicate that more studies should be performed, changing the method of administration, dose, length of treatment and form of administration. There have been many reports on the mecahnism by which IL2 increases the NK cell and LAK cell activities[13,20], and it is thought to be induced most importantly by the increase in the production of γ-interferon[13]. Further investigation is needed to clarify the exact causes of the increased Nk and LAK cell activities.

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