Asian Pacific Journal of Allergy and Immunology (1987) 5 : 13-16

Interleukin 2 and Lymphokine-activated Killer Cells in the Treatment of Childhood Primary Hepatocellular Carcinoma--- A Preliminary Report

Kue-Hsiung Hsieh, Jen-Shin Chang, Hui-Lin Wu* and Chieu-Ts Chu*

SUMMARY **Recombinant interleukin-2 (RIL-2) and lymphokine-activated killer (LAK) cells were administered to 2 boys with the end-stage of primary hepatocellular carcinoma (HCC); the efficacy and toxicity were evaluated. Immunologically, the natural killer and LAK activities were enhanced. Clinically, the side effects were similar to those reported for adults but milder. This kind of treatment may be considered for children with the early stages of hepatocellular carcinoma.**

Although rare in western countries, primary hepatocellular carcinoma (HCC) ranks first as a cause of death from malignancy in Taiwan.[1] Several studies strongly suggest that the high incidence of hepatitis B virus (HBV) infection in Chinese is causally related.[2,3] With the availability of more sophisticated screening techniques, early diagnosis and resection of small HCCs have become possible; moreover, intraarterial embolisation has been successfully used to treat small and localized HCC in certain patients.[4] However, the overall prognosis for HCC remains poor.

Recently, after the availability of recombinant interleukin 2 (RIL-2),[5] lymphokine (IL-2)-activated killer (LAK) cells have been used extensively in the treatment of various human solid tumors with some success.[6-9] In a previous study, we found that LAK cells could be generated in HCC patients and those cells could lyse autologous ($45.8 \pm 6.8\%$) and allogeneic ($48.1 \pm 3.1\%$) fresh HCC tumor cells and several HCC tumor cell lines (greater than 60%).[10] Furthermore, LAK cells had been shown to localize preferentially in the liver several days after intravenous infusions[11] and had been used successfully in the treatment of experimental hepatic metastases.[12,13] We therefore tried to treat 2 boys with primary HCC with RIL-2 and LAK after informed consent was obtained from their parents.

CASE DESCRIPTION

Case 1. A 7-year-old boy was admitted because of epigastralgia, abdominal distension, anorexia and general malaise for 1 month. Physical examination revealed distended abdomen with engorged superficial veins. Liver was palpable 8 cm below RCM with hard coarse nodular surface and spleen 3 cm down to LCM. Breathing sound was vesicular. There was no lymphadenopathy. Abnormal laboratory data included cholesterol 512 mg/dl (normal = 217 mg/dl), ALK-p 20.9 KAU (normal = 10 KAU), SGOT 86 KU (normal = 40 KU), LDH 520 CWU (normal = 450 CWU), α-fetoprotein > 35,000 ng/ml (normal < 20 ng/ml). The patient and his mother were HBsAg-positive and HBeAg-negative. Chest X-rays showed bilateral multiple nodules. Angiography showed marked hepatomegaly and tumor stain. Liver CT scanning showed enlarged liver with multiple cystic masses. Liver biopsy proved to be primary hepatocellular carcinoma with cirrhotic changes. Two successive courses of treatment with RIL-2 and LAK cells were given; however, the disease progressed very rapidly and the child died 3 months after the onset of disease.

Case 2. A 9-year-old boy was admitted because of epigastralgia, abdominal fullness and body weight loss for 3 months. HCC had been

From the Departments of Pediatrics and Bacteriology*, National Taiwan University College of Medicine, Taipei, Republic of China.

Reprint requests to: Kue-Hsiung Hsieh, M.D., Department of Pediatrics, National Taiwan University Hospital, 1 Chang-Teh Street, Taipei, Taiwan 10016; Republic of China.

proved by biopsy at another hospital. On admission, a protruding palm-sized hard mass and several thumb-tip sized nodules were palpable on the right upper quadrant. Abnormal laboratory data included SGOT 78 KU, SGPT 52 KU, LDH 634 CWU and α -fetoprotein > 35,000 ng/ml. The patient and this mother were HBsAg-positive. CT scanning revealed a huge mass on the right lobe and multiple small nodules on both lobes. He received two successive courses of treatment with RIL-2 and LAK cells, but the general condition deteriorated and the patient died 5 months after onset of disease.

METHODS

Preparation of LAK cells

LAK cells were prepared by the method of Rosenberg *et al.*[7] Briefly, peripheral blood mononuclear cells (MNC) were purified by leukapheresis followed by Ficoll/Hypaque density gradient centrifugation. Leukapheresis was done for 5 successive days and 5×10^9 to 1×10^{10} cells were obtained each time. The MNC were adjusted to 1.5×10^6/ml, cultured for 3 days in complete medium (RPMI-1640 supplemented with fetal calf serum and antibiotics) containing 1,000 units RIL-2/ml (Cetus Corp., Emeryville, CA). At the end of cultivation, the resulting LAK cells were washed and finally resuspended in 200 ml of autologous plasma containing 75,000 units of RIL-2.

Administration of LAK cells and RIL-2

The infusion of LAK cells and RIL-2 proceeded according to the protocol of Rosenberg *et al.*[7] with slight modification. One course of treatment consisted of successive infusions of RIL-2 for 10 days and LAK cells for 5 days. RIL-2 at a dosage of 30,000 U/Kg in 20 ml of normal saline was administered intravenously every 8 hours 1 day before transfusion of LAK cells and continued for 4 days after cessation of LAK cell administration. The LAK cells were transfused within a period of one hour and the patient was observed closely during the administration of LAK cells and RIL-2 for possible side effects.

Immunological studies

Blood was drawn every day before starting immunotherapy. Lymphocyte subsets were analyzed by fluorescence-activated cell sorter (FACS 420, Becton-Dickinson). LAK activity against HCC cell lines J5 and J7[14] and natural killer (NK) activity against K562--a myelogenous leukemia cell line--were determined by a 4-hour ^{51}Cr-release assay.[15] Serum immunoglobulin concentrations were checked using immunoplates (Behring, West Germany). IL-2 titration was done according to Gillis *et al.*[16]

RESULTS AND DISCUSSION

Clinical observation

Moderate to high fever, chilliness and headache were the usual complaints after the completion of RIL-2 infusion. Itching maculopapules were found from the 4th day of the treatment. Those symptoms were tolerable, and could be controlled by indomethacin and antihistamine. Hair loss, decreased urine volume and mild lower leg edema developed at the end of the first course and worsened gradually until discontinuation of treatment. Precordial compressive sensation with air hunger developed each time during the infusion of LAK cells. Anemia and hypoproteinemia developed from the 5th day of treatment, and may have been due to repeated leukapheresis. Transient increase of BUN (57.2 mg/dl), creatinine (2.9 mg/dl) and

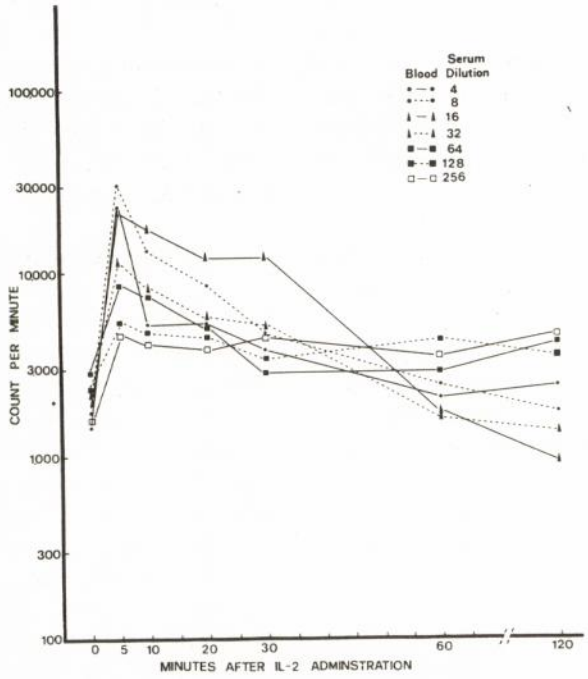

Fig. 1 Serial determinations of IL-2 in sera of patients after bolus infusion of 30,000 U/kg of RIL-2. Serum activity was measured in specimens heated at 56°C for 30 minutes in the standard assay.[16] Only slight activity was demonstrable 2hr after infusion. Each point represents the mean of 2 boys.

total bilirubin (1.6 mg/dl) were also noted. α-fetoprotein remained very high at the end of the second course of treatment.

IL-2 activity after intravenous (I.V.) administration

Blood was drawn serially after RIL-2 adminstration; the serum activity was determined by the method of Gillis *et al,*[16] after the serum was heated at 56°C for 30 minutes. Fig. 1 illustrates that 2 hours after I.V. infusion, only slight IL-2 activity could be detected. Thus the serum IL-2 activity after I.V. infusion disappeared much quicker than has been reported for adults.[17] The increase in urine IL-2 activity after dilution up to 64-fold or higher suggests that there may be an IL-2 inhibitor in urine.

The serial changes of immunological parameters are shown in Table 1. The total numbers of lymphocytes, T lymphocytes (OKT3$^+$ cells) and NK cells (Leu 11b$^+$ cells) were much decreased during the first 2 days of regular (*q*.8 h.) IL-2 infusion. All types of cells rebounded up to more than 2-fold the original number around the 5th to 7th day, and then gradually returned to pretreatment levels in spite of continuous IL-2 administration. The mitogenic responses to PHA, Con A and PWM decreased markedly until the 8th day. These immunological changes were largely in agreement with those reported by Lotze *et al.*[17] and recirculation of lymphoid cells induced by IL-2 was suggested to be the cause. It is interesting to note that total eosinophil count and serum IgG increased gradually after I.V. infusion of IL-2. The increased IgG may be due to the effect of IL-2 on B cell differentiation,[18] but the me-

Table 1 Cellular changes after treatment of LAK cells and recombinant interleukin 2 in two boys with primary hepatocellular carcinoma

	Day after treatment										
	0	1	2	3	4	5	6	7	8	9	10
Total lymphocyte (/mm^3)	2,541	363	365	2,604	3,584	5,940	6,118	3,780	2,142	2,352	2,484
Total T lymphocyte (OKT3$^+$, /mm^3)	1,600	122	95	1,352	2,000	2,274	2,550	1,620	963	987	1,023
Natural Killer cells (Leu 11b$^+$, /mm^3)	457	23	144	850	1,040	1,473	2,121	1,058	771	872	895
Mitogen response (cpm)											
PHA	318,484			98,433	45,950		50,792	121,777		399,453	267,393
Con A	479,647			12,636	20,971		141,458	141,466		205,839	142,522
PWM	110,734			6,100	34,766		31,621	17,276		89,319	64,171
Control	9,399			4,437	45,764		20,209	22,680		27,871	35,075
IgG (mg/dl)	770	463	908	555	1,070	1,020	1,190		1,860	1,760	1,970
Total eosinophil count (/mm^3)	134	297	280	1,388	1,518	1,235	687	673	950	984	603

All figures represent the mean of 2 patients.

Table 2 NK and LAK activities* before and after recombinant interleukin 2 treatment

I.V. infusion of interleukin-2 for 10 days	Target								
	K562			J5			J7		
	LAK	CM	Fresh MNC	LAK	CM	Fresh MNC	LAK	CM	Fresh MNC
Before	ND	ND	32.2%	22.3%	ND	4%	9.0%	ND	7%
After	89%	ND	59.0%	46.0%	ND	13%	61.0%	9.9%	19%

* K562 (NK target) and J5 and J7 (HCC cell lines as LAK targets) were labelled with ^{51}Cr. LAK cells were generated by incubating mononuclear cells (MNC) with RIL-2 (1000 units/ml) for 3 days. All cytotoxicity assays were done in triplicate and at an effector: target ratio of 50:1.
ND: not done. CM: culture medium.
All figures represent the mean of 2 boys.

chanism of eosinophilia is difficult to explain because continuous IL-2 administration augmented the secretion of ACTH and increased serum cortisol,[8] and both will cause eosinophilopenia.

The most important finding of this study was that, after continuous bolus I.V. infusion of IL-2 for 10 days, the NK and LAK activities were markedly enhanced (Table 2). As both NK and LAK cells are involved in tumor killing, it is speculated that, if IL-2 and LAK cells are given locally through the hepatic artery or are combined with intra-arterial embolisation, or are administered systemically after surgical resection of small tumors, the prognosis of patients with small hepatocellular carcinoma should be markedly improved. Larger scale phase II study is now underway.

REFERENCES

1. Department of Health, Executive Yuan; Department of Health, Taiwan Provincial Government; Taipei City Health Bureau; General Health Statistics, 1977; p 54-6.
2. Tong MT, Sun SC, Scheaffer BT. Hepatitis-associated antigen and hepatocellular carcinoma in Taiwan. Ann Int Med 1975; 75: 687-9.
3. Beasley RP, Hwang LY, Lin C. Hepatocellular carcinoma and HBV: prospective study of 22,707 men in Taiwan. Lancet 1981; 2:1129-33.
4. Wheeler PG, Melia W, Dubbis P, *et al.* Non-operative arterial embolization in primary liver tumor. Br Med J 1979; 2:242-4.
5. Rosenberg SA, Grimm EA, McGrogan M, *et al.* Biological activity of recombinant human interleukin 2 produced in *Escherichia coli.* Science 1984; 223:1412-5.
6. Pizza G, Severini G, de Vinci C, Corrado F. Tumor regression after intralesional injection of interleukin 2 (IL-2) in bladder cancer. Preliminary report. Int J Cancer 1984; 34:359-67.
7. Rosenberg SA, Lotze MT, Muul LM, *et al.* Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 1985; 313: 1475-92.
8. Lotze MT, Rosenberg SA. Treatment of tumor patients with purified human interleukin-2. In: Sorg C, Schimpl A, eds, Cellular and molecular biology of lymphokines. New York: Acad Press, 1985: 711-9.
9. Mule JJ, Rosenberg SA. Immunotherapy of cancer with lymphokine activated killer cells and recombinant interleukin 2. Surgery 1985; 98: 437-44.
10. Hsieh KH, Shu S, Lee CS, *et al.* Lysis of primary hepatic tumors by lymphokine-activated killer cells. Gut 1987; 28:117-24.
11. Lotze MT, Line BR, Mathisen DJ, Rosenberg SA. The in vivo distribution of autologous human and murine lymphoid cells grown in T cell growth factor (TCGF). Implication for the adoptive immunotherapy of tumors. J Immunol 1980; 125: 1487-93.
12. Lafreniere R, Rosenberg SA. Successful immunotherapy of experimental hepatic metastases with lymphokine-activated killer cells and recombinant interleukin 2. Cancer Res 1985; 45: 3735-40.
13. Lafreniere R, Rosenberg SA. Adoptive immunotherapy of murine hepatic metastases with lymphokine-activated killer (LAK) cells and recombinant interleukin 2 (RIL-2) can mediate the regression of both immunogenic and non-immunogenic sarcomas and an adenocarcinoma. J Immunol 1985; 135: 4273-80.
14. Chang K SS, Wang LC, Yang CS. Studies on human hepatocellular carcinoma cell lines. Karyotype, retrovirus replication, and cellular oncogenes. Proceedings of the Taipei Pre-congress Symposium of the Sixth International Congress of Virology, Taipei, Republic of China, Chinese Society for Microbiology, Aug. 27-29, 1984: p 17.
15. Grimm EA, Mazumder A, Zhang HI, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med 1982; 155: 1823-41.
16. Gillis S, Ferm M, Ou W, Smith KA. T cell growth factor: Parameters of production and a quantitative microassay for activity. J Immunol 1978; 120: 2027-32.
17. Lotze MT, Matory YL, Ettinghausn SE, *et al.* In vivo administration of purified human interleukin 2. II. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL-2. J Immunol 1985; 135: 2865-75.
18. Mayer L, Crow MK, Thompson C. Synergy between B cell differentiation factors and interleukin 2, using a monoclonal system. J Immunol 1985; 135: 3272-6.