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New Immunotherapy Methods
I. T Cell Growth Factor (IL-2) Administered by Using Transfusion Bags, Augmented Lymphokine Production

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新しい方法による免疫療法
Ⅰ．輸血バックを使ったT細胞増殖因子（IL-2）の投与によるリンホカイン産生の増強

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近年の研究によって，分化したT細胞が持続的に増殖するためには，T細胞増殖因子(IL-2と呼ばれる)が必要なことが明らかとなった．そしてIL-2をIn vivo投与することによって，免疫不全の治療や抗腫瘍免疫の増強を計ることも試みられている．その際IL-2が機能を発揮するためには，まず受容体を持つ細胞（分化したT細胞）と結合する必要があり，そのためにはIL-2の至適濃度が細胞周囲に一定時間保たれていなければならない．しかし今迄の報告ではいずれも静注，腹腔投与などが行なわれており，IL-2は投与後速やかに稀釈されている．そして結果的にも一定の効果が得られていない．我々はこの研究において，IL-2を腫瘍免疫療法に役立てるために，輸血バッグを用いて投与する方法を開発した．この方法を使えば，IL-2は分化したT細胞に確実に結合した上で投与されることが確かめられた．したがってその後体内で機能T細胞が増殖すれば，腫瘍抗原も含めた色々な抗原にたいする免疫反応が増強されることが想定された．今回は患者の腫瘍細胞が得られなかったためIL-2の腫瘍免疫反応への影響は調べられなかった．しかしそのIn vivo効果を治療として使われた細菌性免疫賦活製剤（OK432）に関連したT細胞機能により調べたところ，いくつかのリンホカイン産生の増強が検出された．これにより投与されたIL-2は患者体内で機能を発現し得たこ

とが確かめられた.

近年，遺伝子操作によりこの治療に必要な量の組換え型IL-2（例えばTGP-3，武田製薬）も入手可能となって来ている．我々の方法によるIL-2の投与は，単独でも患者の特異的抗腫瘍免疫反応を増強出来る可能性をもつとともに，免疫賦活剤との併用や，この研究でも試みられたLAK（リンホカイン活性化キラー）細胞との併用による非特異的受動免疫療法などの新しい腫瘍治療となり得ることが考えられる.

Introduction

T cell growth factor (IL-2), a lymphokine capable of sustaining the long-term growth of thymus-derived lymphocytes (T cells), is secreted by activated T cells and plays an important requlatory role in the T cell response to an antigen[1]. T cells require activation by a specific antigen or lectins for the expression of IL-2 receptors on the cell surface. Recent studies show that natural killer (NK) cells also respond to IL-2[2]. Very little is, however, known concerning the use of IL-2 in vivo as a pharmacologic agent. When injected intravenously, IL-2 is known to be rapidly cleared from the circulation in mice and humans and it's therapeutic effect was equivocal[3]~[5]. In this study, partially purified, lectin-free human IL-2 was repeatedly administered to a tumour patient using transfusion bags, to allow IL-2 to bind to receptor bearing cells. The effect of exogenous IL-2 on T cells in vivo was analysed by a time course study of T cell counts and lymphokine production. With our new administration method, exogenous IL-2 was shown to augment the T cell function and possibly modulate the numbers of T subpopulation cells in the peripheral blood.

Materials and Methods

Patient. A 54-year-old man (T.Y) was admitted at the Department of Radiology, Kyusyu University on May 7, 1983 because of nasopharyngeal carcinoma with bone metastasis. An excisional biopsy of the tumor revealed squamous cell carcinoma. The primary tumour was cured by combined radiotherapy and chemotherapy, and no local recurrence had been observed thereafter. He was discharged on Dec. 16, 1983. He was re-hospitalized on Jan. 19, 1984, because of severe back pains due to extensive bone metastasis. The disseminated disease was persistently refractory to chemotherapy and he suffered from the severe side effects of the drugs, i.e vomiting, nausea, kidney dysfunction, etc.. Then, in agreement with the patient and his family, immunotherapy was scheduled on May 9, which consisted of daily intradermal injections of OK432 (0.2KE), and intravenous administration of OK432 (0.1KE), exogenous IL-2 and autologous lymphokine activated killer (LAK) cells as shown in Table 1. The patient had less pains during the immunotherapy, but serial scintiphotoscanning of the bones with ^{99m}Tc-MDP did not reveal any regression nor progression of the bone metastasis. He died of subdural hemorrhage on June 18, 1984. Autopsy was not carried out.

Table 1 Time relationship between IL-2 administration and laboratory examinations.

	May								June				
Date	2	18	22	24	25	28	29	31	5	7	9	12	14
IL-2 administration				(1) 5000units IL-2 +0.1KE OK432			(2) 5000units IL-2 +1×10⁸LAk cells	(3) 5000units IL-2 +1×10⁸LAk cells	(4) 5000units IL-2 +1×10⁸LAk cells	(5) 5000units IL-2 +1×10⁸LAk cells			
Blood transfusion	(1)*										(2)		(3)
T cell subsets analysis	(1)						(2)		(3)			(4)	
Ab titration to OK432	(2)				(3)				(4)				(5)
LMI assay	(2)				(3)			(4)	(5)	(6)		(7)	
Lymphotoxin assay		(1)		(2)			(3)	(4)	(5)	(6)		(7)	(8)
Interferon-γ assay		(1)			(2)		(3)		(4)				(5)

*The numbers in parentheses indicate the order of treatments or examinations.

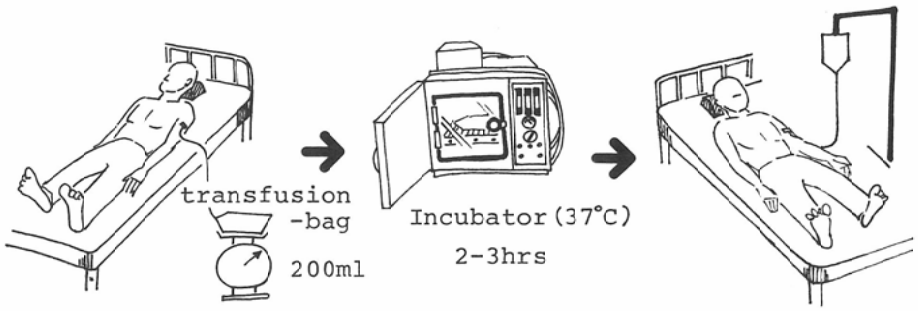

Fig. 1 Administration method of IL-2, using a transfusion bag.

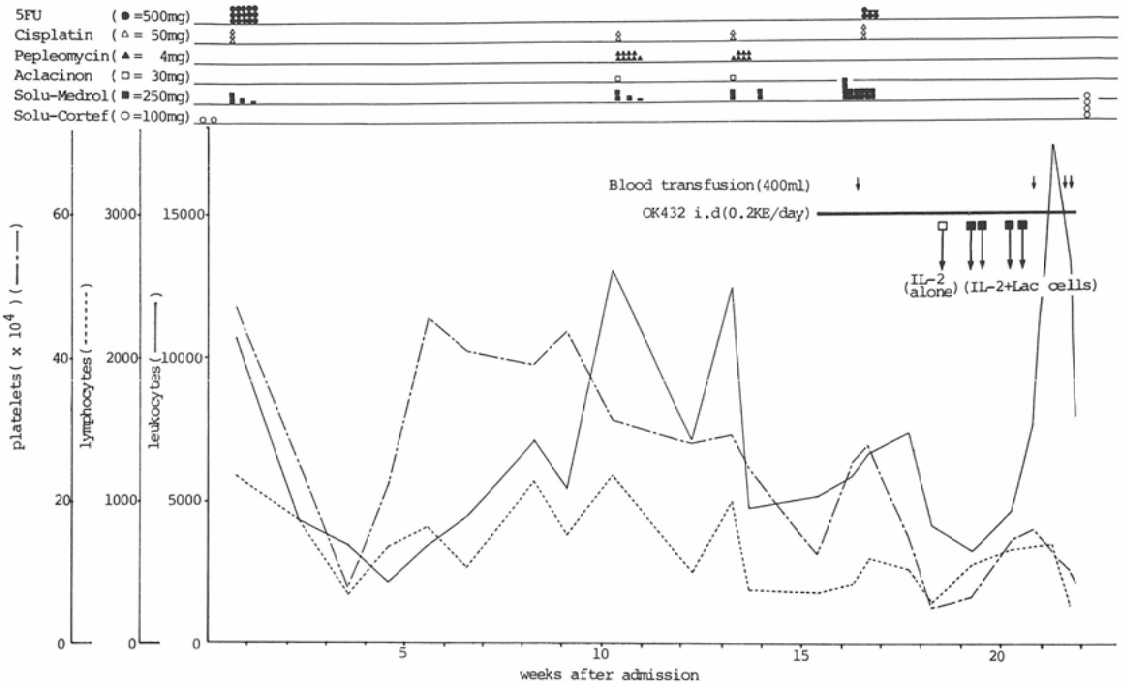

Fig. 2 Clinical course of the patient.

Immunopotentiator. OK432 (Picibanil, Chugai pharmaceutial Co. Ltd., JAPAN) is a lyophilized preparation of an attenuated strain of Streptococcus haemolyticus. It was dissolved in physiological saline to an appropriate concentration before use. 0.2KE (200 μg protein dose) in 0.2 ml Xylocain was intradermally injected in the supraclavicular, axillary, parasternal, inguinal and forearm areas, which were predetermined in a chronological order[6].

Preparation of IL-2. IL-2 was derived from the supernatant of Con A-stimulated pooled peripheral blood lymphocytes from healthy donors[7]. Lymphocytes were cultured for 2 hours at 37°C with Con A (5μg/ml) (Pharmacia Fine Chemicals, Sweden), washed once, and then further cultured for 48 hrs to collect the cell-free culture supernatant. This crude IL-2 preparation was then partially purified by Gel-filtration[8]. IL-2

content was assayed by the method of Gillis using a standard human IL-2 (GUPI-2, Genzyme U.S.A.)[9]. This IL-2 preparation was sterilized by using 0.22 μm filter units (Millipore Corporation, U.S.A.) and stored at −80°C until use.

Preparation of LAK cells. Patient's peripheral blood lymphocytes at 1×10^6/ml were cultured with Con A (5 μg/ml) for 3 days. The cells were washed and live cells were separated using Ficoll-Conray (density 1.077—1.080 g/ml). The cells were further cultured for 2—3 weeks in IL-2 conditioned RPMI1640 medium/10% FCS/15 units IL-2/ml, changing the medium every 3 days. The cells were then harvested and used either for immunotherapy or as effector cells for lysis of cryopreserved allogeneic lung carcinoma cells [2 adenocarcinomas (M.H and T.T), 1 small cell carcinoma (K.K) and 1 squamous cell carcinoma (QG-56)] or autologous lymphocytes. Cytotoxicity of LAK cells was tested in 6 hr and 18 hr ^{111}In-release assay at 40:1 of the effector/target ratio[10]. Data is reported as percent specific lysis which is calculated as follows:

$$\% \text{ specific lysis} = \frac{\text{cpm of test well} - \text{cpm of control well}}{\text{total cpm incorporated into cells}} \times 100$$

Administration of IL-2. 200 ml blood was retracted into a transfusion bag (TERUMO BB-SA206J01, JAPAN) following the manufacturer's instructions. To that, 5000 units of purified IL-2, together with 1×10^8 LAK cells, were added. The mixture was then incubated for 2—3 hrs at 37°C (Fig. 1). After the incubation, the blood was transfused to the patient in an ordinary manner. The administration schedule is shown in Table 1 and Fig. 2.

Autoradiography. Purified IL-2 was radiolabelled with Na ^{125}I by using Borton Hunter reagents (Amersham, UK), according to manufacturer's instructions. 0.5 μCi of ^{125}I-IL-2 was added to 1×10^6 LAK cells and the mixture was then incubated for 5 hrs. At various time points, cells were harvested from the cultures to prepare autoradiograms[11].

Assays of lymphokine. Lymphotoxin, leukocyte migration inhibitory factor and interferon-γ for the assays were prepared from the peripheral blood lymphocytes of the patient. Lymphocytes were adjusted to 1×10^6 cells/ml, and 5 μg/ml OK432 were added to the cell suspensions. The culture supernatants were collected after 48 hrs to obtain leukocyte migration inhibitory factor and interferon-γ, and after 72 hrs to obtain lymphotoxin.

Lymphotoxin activity was assayed by the method of Kolb et al.[12], with minor modifications using mouse L cells[13]. Interferon-γ activity was measured using the dye-uptake method of Imanishi[14]. Leukocyte migration inhibition assay was performed based on Clausen's modified indirect agarose plate technique[15], wherein a migration inhibition index (MI) of less than 85 was chosen as positive inhibition.

T subsets analysis. Surface phenotypes of T cells were determined by using monoclonal antibodies to T cell antigens (Ortho pharmaceutical corporation, U.S.A.) and a fluorescein conjugated anti-mouse immunoglobulin antibody as the second antibody. The cells were analysed by flow cytofluorometry using a Ortho Spectrum III (Ortho Diagnostic Systems Inc. U.S.A.).

Detection of antibody to OK432. Serum antibody to OK432 was measured by passive hemagglutination as previously described[16].

Results

In vitro growth and cytotoxicity of LAK cells

Con A stimulated patient's peripheral blood lymphocytes grew 2—3 times in the IL-2 conditioned medium every 3 days (data not shown). LAK cells thus prepared could lyse allogeneic lung tumour cells, but not autologous lymphocytes: % cytotoxicity against 4 allogeneic tumour cells in 18 hr assay averaged 26.1 ± 9.4% (Table 2) and against autologous lymphocytes in 6 hr assay, 1.5 ± 0.9%. Fresh peripheral blood lymphocytes from the patient did not express any cytotoxicity against these tumour cells in the same assay.

Binding of IL-2 to activated T cells

Table 2 Cytotoxicity of LAK cells from the patient(T.Y) against allogeneic tumour cells

tumour cell \ LAK cell	T.Y
M.H	20.3±5.5*
T.T	26.5±3.4
K.K	39.2±4.2
QG-56	18.4±2.3
average	26.1±9.4

* Cytotoxicity of LAK cells is expressed by percent specific lysis in a 18 hr assay. The effector to target cell ratio was 40 : 1. Each value is the mean±SE of quadricate determinations.

Table 3 T cell subsets analysis*

Examination No.[1]	Before IL-2 treatment	During IL-2 treatment		After IL-2 treatment	Normal value
	1	2	3	4	
peripheral blood lymphocytes/mm	546	544	644	692	1622±612[3]
OKT3 (pan T cells)[4]	43.0%(235)[2]	31.6%(172)	33.2%(214)	48.7%(337)	69.5±6.1(%)
OKIa 1 (activated T cells)[4]	18.7%(102)	20.5%(112)	23.6%(152)	23.1%(160)	17.6±6.7(%)
OKT10 (activated T cells)[4]	46.2%(252)	61.7%(336)	64.3%(414)	34.8%(241)	12.3±5.7(%)
OKT4 (helper/inducer T cells)[4]	24.7%(135)	15.3%(83)	13.3%(86)	30.3%(210)	38.1±6.9(%)
OKT8 (killer/suppressor T cells)[4]	23.4%(128)	22.0%(120)	29.5%(190)	27.5%(190)	28.8±6.5(%)
4/8 ratio	1.06	0.70	0.45	1.10	1.35±0.45

*Carried out by Special Reference Laboratories, Inc.(Tokyo, JAPAN)

1) See Table 1.

2) Number in parenthesis indicates the absolute cell number.

3) Normal values of individuals 50-60 years of age.

4) % of cells in the peripheral blood lymphocytes.

We examined the binding ability of our IL-2 preparation to the receptor-bearing cells by using the above mentioned LAK cells. The binding of an appreciable amount of IL-2 already occurred after 30 minutes and maximum binding was attained after 1—2 hr incubation (Fig. 3). 2 hr incubation time to coat the cells with IL-2 in a transfusion bag was then determined by this results.

The effects of exogenous IL-2 on the T cell number and function

Total T cell counts (OKT3$^+$ cells) in the peripheral blood lymphocytes remained unchanged. However, the numbers of subpopulation T cells were changed by the IL-2 administration: OKT4$^+$ cells decreased and OKT8$^+$, OKT10$^+$ and OKIal$^+$ cells increased, and as a consequence OKT4/8 ratio diminished (Table 3).

Since no autologous tumour cells for the assay was available, IL-2 effect on the T cells as to tumour immunity could not be examined. IL-2 effect on the T cell functions was then assessed in relation to immunity to OK432, the patient having been treated with the agent on a continuous basis. The ability to produce lymphokines upon stimulation with OK432 was examined. Among the lymphokines examined, lymphotoxin and leukocyte migration inhibitory factor production were significantly enhanced (Figs. 5 and 6), while in-

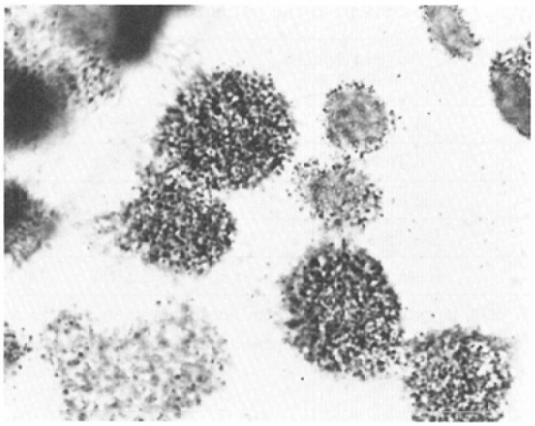

Fig. 3 Microautoradiogram of LAK cells, showing the binding of ^{125}I-IL-2 after 2 hr incubation (original magnigication 1400×).

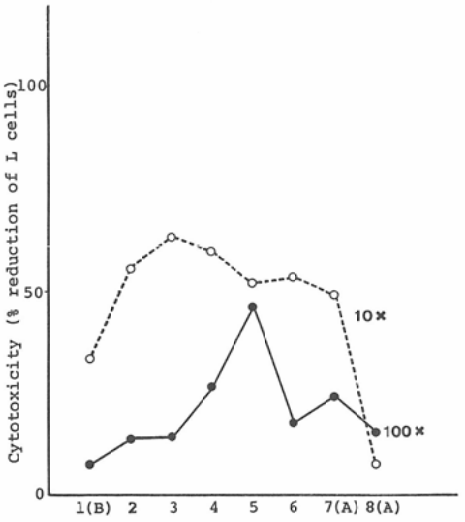

Fig. 4 Lymphotoxin production by peripheral blood lymphocytes with OK432 (5 μg/ml). (B) and (A) on the abscissa denote before and after the IL-2 administration respectively. The numbers 1—8 denote the chronological order of the examinations as shown in Table 1.
(○---○ 10× dilutions and ●—● 100× dilutions in the assay).

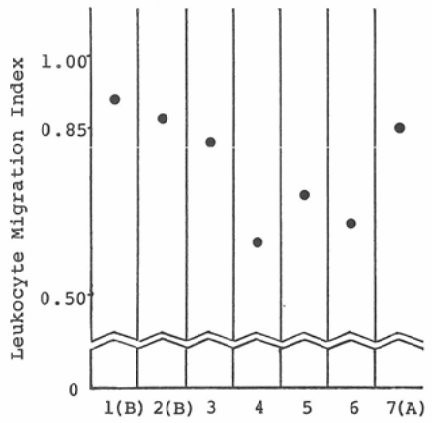

Fig. 5 Leukocyte migration inhibition with OK432 tested before, during and after the IL-2 admiistration. Legends (B), (A) and 1 to 7 are similar to that in Fig. 4.

terferon-γ production, which was at 125 units/1 × 10^6 cells before the administration, was completely abolished by the exogenous IL-2 (data not shown). Any detectable antibody to OK432 was not produced in this patient throughout the immunotherapy. Thus, administration of IL-2 had no effect on the antibody response, wherein helper T cell function can be measured.

Discussion

The half-life of IL-2 after intravenous injection is extremely short. It was about 20 minutes in 2 patients by Bindon[4]. The initial levels of IL-2 in the circulation of these two patients was related to the dose given, and 5000 units, which was the same dose used in this study, gave an initial level of 1 unit/ml. Such a low concentration did not support the growth of LAK cells in our study. By using a 200 ml transfusion bag, higher levels of IL-2 (25 units/ml blood) can be maintained until the maximum binding of IL-2 to the receptor bearing cells occurs. Then through this method, any unbound IL-2 is also administered and it's therapeutic effect can be also made use of. The binding of our IL-2 preparation to the activated T cells (LAK cells) was confirmed by autoradiography. Thus, our new administration method seems more promising in effecting IL-2 in vivo.

Exogenous IL-2 augmented the ability of T cells to produce lymphotoxin and leukocyte migration inhibitory factor by the stimulation with a streptococcal antigen, OK432. This finding suggests that exogenous IL-2 also augments T cell functions related to various antigens, including tumour associated antigen(s). This assumption may also be supported by the findings that addition of IL-2 overcomes various immunosuppressed conditions[17)~19)].

OK432 induces both humoral and cellular immune responses in animals and humans. We previously studied the anti-tumour effect of OK432 in relation to the immune responses to the agent[16]. The anti-tumour effect of OK432 correlated to the cellular immune response. Among the several mechanisms of the anti-tumour activity of OK432, one is most probably mediation by lymphokines[20)21)]. It has been shown that, upon stimulation with OK432, primed T cells release various mediators (lymphokines), some of which act directly or indirectly on the tumour cells, eventually causing damage to these tumour cells. Lymphotoxin and interferon are such examples with a direct action. On the other hand, leukocyte migration inhibitory factor requires one more step before expressing it's anti-tumour activity, i.e., leukocytes, upon interaction with the factor, have less active migratory activity and as a consequence accumulate at the site. Although several reports indicate that leukocytes, especially neutrophils, accumulate at the tumour site, cytotoxicity of the cells on tumour cells has not been well established[22]. However, it was shown in a patient that a large number of neutrophils accumulated in the peritoneal cavity and remarkably damaged ascitic tumour cells after intraperitoneal injection of OK432[23]. In this study, lymphotoxin and leukocyte migration inhibitory factor productions with OK432 were remarkably enhanced by exogenous IL-2. This finding suggests that exogenous IL-2 is useful in augmenting the effect of immunotherapy with OK432 as well.

The reason why interferon-γ was suppressed by exogenous IL-2 cannot be definitely ascertained. One possible explanation is that lymphokines are produced by different subpopulations of T cells, interferon-γ being mainly produced by $OKT4^+$ cells, since both interferon-γ and $OKT4^+$ cells concurrently decreased during exogenous IL-2 administration in this study.

Lymphokine activated killer cells (LAK cells), after longer periods of culture with IL-2, become lytic against fresh autologous as well as a wide range of allogeneic solid tumour cells[24]. When such LAK cells are administered with IL-2, they are assumed to grow further in vivo in an IL-2 dependent manner expressing killer cell activity. Further analysis of LAK cells demonstrated that cytotoxicity of LAK cells is mediated both by activated T cells and by natural iller cells (NK cells)[2]. To gain more therapeutic effect, LAK cells prepared from the patient's peripheral blood lymphocytes were administered with IL-2, after confirming that they were not cytotoxic to his own lymphocytes.

^{111}Indium-oxin chelate ([^{111}In]OX) has not yet been commonly used in isotope release assays. [^{111}In]OX, however, has several advantages over other radiolabels, i.e., ^{51}Chromium (^{51}Cr), [^{125}I] iododeoxyuridine ([^{125}I]dUrd) or tritiated thymidine ([^{3}H]TdR). [^{111}In]OX efficiently labeled both adherent (tumour cells) and non-adherent cells (lymphocytes) with no decrease in cell viability. [^{111}In]OX localized in the cytoplasm and the incorporated label undergoes very slow spontaneous release, while being rapidly released upon im-

munemediated target cell destruction. Released [^{111}In]OX is not reutilized[10]. [^{111}In]OX is a γ-emitter which does not require an extensive sample preparation for liquid scintillation, as ^{51}Cr or ^{3}H, β-emitters, does. With tumour target cells, after 18 hr incubation, highest levels of target cell destruction were observed, as assessed by [^{111}In]OX-release. However, with lymphocyte target cells, whose spontaneous release of [^{111}In]OX was higher than that of the tumour cells, the 6 hr ^{111}In-release assay was found to be adequate to detect cell destruction. Further investigations are required for establishing [^{111}In]OX-release assay.

IL-2 can be repeatedly administered without any serious side effects. There had been no abnormal findings in clinical and laboratory examinations. The only complaint with the patient was a chill during the first 3 injections, which completely disappeared thereafter. Fever, reported by others as a side effect, was not evident in this case[4].

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References

1) Morgan, D.A., Ruscetti, F.W. and Gallo, R.C.: Selective in vitro growth of T-lymphocytes from normal human bone marrows. Science 193: 1007—1008, 1976
2) Henney, C.S., Kuribayashi, K., Kern, D.E. and Gillis, S.: Interleukin-2 augments natural killer cell activity. Nature 291: 335—338, 1981
3) Donohue, J.H., Lotze, M.T., Robb, R.J., Rosenstein, M., Braziel, R.M., Jaffe, E.S. and Rosenberg, S.A.: In vivo administration of purified Jurkat-derived interleukin-2 in mice. Cancer Research 44: 1380—1386, 1984
4) Bindon, C., Czerniecki, M., Ruell, P., Edwards, A., McCarthy, W.H., Harris, R. and Hersey, P.: Clearance rates and systemic effects of intravenously administered inrerleukin-2 (IL-2) containing preparations in human subjects. Br. J. Cancer 47: 123—133, 1983
5) Cheever, M.A., Greenberg, P.D., Irle, C., Thompson, J.A., Urdal, D.L., Mochizuki, D.Y., Henney, C.S. and Gillis, S.: Interleukin-2 administered in vivo induces the growth of cultured T cells in vivo. J. Immunol. 132: 2259—2265, 1984
6) Makidono, R. and Matsuura, K.: Immunologic capacity of tumour patients and possible immunotherapy. Jpn. J. Cancer Clin. 24: 965—971, 1978
7) Spiess, P.J. and Rosenberg, S.A.: A simplified method for the production of murine T-cell growth factor free of lectin. J. Immunol. Methods 42: 213—222, 1981
8) Gillis, S., Smith, K.A. and Watson, J.: Biochemical characterization of lymphocyte regulatory molecules II. Purification of a class of rat and human lymphokines. J. Immunol. 124: 1954—1962, 1980
9) Gillis, S., Ferm, M.M., Ou, W. and Smith, K.A.: T cell growth factor: Parameters of production and a quantitative microassay for activity. J. Immunol. 120: 2027—2032, 1978
10) Wiltrout, R.H., Taramelli, D. and Holden, H.T.: Measurement of macrophage-mediated cytotoxicity against adherent and non-adherent target cells by release of ^{111}Indium-Oxin. J. Immunol. Methods 43: 319—331, 1981
11) Rogers, A.W.: Techniques of autoradiography 1967, Elsevier, Amsterdam
12) Kolb, W.P., Williams, T.W. and Granger, G.A.: Lymphocyte activation and lymphotoxin production. In Vitro Methods in Cell-Mediated Immunity. ed. Bloom, B.R. and Glade, P.R., pp. 333—342, 1971, Academic Press, New York.
13) Gojobori, M., Mashiba, H., Matsunaga, K. and Jimi, S.: Lymphotoxin production in cancer patients: Possible relation to blastogenesis and the degree of tumour disease. Gann 69: 201—206, 1978
14) Imanishi, J., Hoshino, S., Hoshino, A., Oku, T., Kita, M. and Kishida, T.: New simple dye-uptake assay for interferon. Biken Journal 24: 103-108, 1981
15) Clausen, J.E.: Tuberculin-induced migration inhibition of human peripheral leukocytes in agarose medium. Act. Allergol. 26: 56—80, 1971
16) Makidono, R., Matsuura, K. and Makidono, A.: Anti-tumour effect of a streptococcal preparation (OK-432) 1. Positive co-relation between the anti-tumour effect and delayed type hypersensitivity reaction to OK-432 in mice. Nippon Act. Radiol. 25: 379—390, 1984

17) Kruger, G., Welte, K., Ciobanu, N., Cunningham-Rundles, C., Ralph, P., Venuta, S., Feldman, S., Koziner, B., Wang, C.Y., Moore, M.A.S. and Mertelsmann, R.: Interleukin-2 correction of defective in vitro T-cell mitogenesis in patients with common varied immunodeficiency. J. Clin. Immunol. 4: 295—303, 1984
18) Flomenberg, N., Welte, K., Mertelsmann, R., Kernan, N., Ciobanu, N., Venuta, S., Feldman, S., Kruger, G., Kirkpatrick, D., Dupont, B. and O'Reilly, R.: Immunologic effects of interleukin-2 in primary immunodeficiency diseases. J. Immunol. 130: 2644—2650, 1983
19) Emery, P., Panayi, G.S. and Nouri, A.M.E.: Interleukin-2 reverses deficient cell-mediated immune responses in rheumatoid arthritis. Clin. exp. Immunol. 57: 123—129, 1984
20) Saito, M., Yamaguchi, T., Ebina, T., Koi, M., Aonuma, E., Usami, H. and Ishida, N.: In vitro production of immune interferon (IFN) by murine spleen cells when different sensitizing antigens are used in vivo and in vitro. Cell. Immunol. 78: 379—386, 1983
21) Wakasugi, H., Kasahara, T., Minato, N., Hamuro, J., Miyata, M. and Morioka, Y.: In vitro potentiation of human natural killer cell activity by a streptococcal preparation, OK-432: Interferon and interleukin-2 participation in the stimulation with OK-432. J.N.C.I. 69: 807—812, 1982
22) Dvorak, A.M., Connell, A.B., Proppe, K. and Dvorak, H.F.: Immunologic rejection of mammary adenocarcinoma (TA3-St) in C57BL/6 mice: Participation of neutrophils and activated macrophages with fibrin formation. J. Immunol. 120: 1240—1248, 1978
23) Katano, M. and Torisu, M.: Neutrophil-mediated tumour cell destruction in cancer ascites. Cancer 50: 62-68, 1982
24) Grimm, E.A., Robb, R.J., Roth, J.A., Neckers, L.M., Lachman, L.B., Wilson, D.J. and Rosenberg, S.A.: Lymphokine-activated killer cell phenomenon. III. Evidence that IL-2 is sufficient for direct activation of peripheral blood lymphocytes into lymphokine-activated killer cells. J. exp. Med. 158: 1356—1361, 1983