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(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF B-CELL MALIGNANCIES

(57) Abstract

The present invention concerns pharmaceutical compositions for treating B-cell malignancies in a mammal afflicted with B-cell malignancies. More precisely, according to the invention, these pharmaceutical compositions comprise IL-4 as an active ingredient. The present invention is also directed to a method of treating B-cell malignancies, or inhibiting the proliferation or growth of malignant B-cell by administering IL-4 into mammals afflicted with B-cell malignancies.

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PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF B-CELL MALIGNANCIES

BACKGROUND OF THE INVENTION

This invention relates to pharmaceutical compositions comprising IL-4 as an active ingredient for the treatment of B-cell malignancies in a mammal afflicted with B-cell malignancies. The invention further relates to the use of IL-4 for making medicaments for treating B-cell malignancies in mammals.

INTRODUCTION

Interleukin-4 [hereinafter "IL-4" but also known as B Cell Stimulatory Factor 1, (BSF-1)] was originally described by M. Howard et al. in J. Exp. Med. (1982), Vol. 155, pp. 914-23 as a T cell-derived growth factor, distinct from IL-2, which permitted long-term tissue culture of normal mouse B lymphocytes and which interacted with activated B lymphocytes to maintain the proliferation thereof for as long as 4 months. Although mixed B lymphocyte explants have been used to initiate cultures, it appears that B lymphocytes with immature phenotype are specifically enhanced by IL-4 in tissue culture. See for example C. Peschel et al., J. Immunol. (1989), Vol. 142, 1558-1568. In addition, G. Trenn et al. J. Immunol. (1988) Vol. 140, 1101-1106 discloses that IL-4 stimulates the development of cytotoxic T cells from the Lyt-2+ subpopulation of resting murine T lymphocytes.

The mouse IL-4 gene was cloned and expressed in COS-7 cells [See T. Otsuka et al., Nuc. Acids Res. (1987), Vol. 15, 333-334]. The cloned factor had all the activities in tissue culture seen for the factor purified from T cell culture supernatants. Cloning and expression of the human IL-4 gene have been described by

N. Arai et al., J. Immunol. (1989, Vol. 142, 274-282 and T. Yokota et al., Proc. Natl. Acad. Sci. (1986), Vol. 83, 5844-5848 with the factor produced in COS-7 cells having similar activities to the native molecule as studied in tissue culture. As IL-4 was studied both in human and murine cell systems, additional in-vitro activities were attributed to the molecule: (i) IL-4 played an important role in the induction and regulation of IgE synthesis, a process occuring as B lymphocyte subpopulations were induced into proliferation [See Pene, J., Proc. Natl. Acad. Sci (1988), Vol 85, 6880-6884]; (ii) IL-4 induced low affinity FcΣ receptors (CD23) on normal human B lymphocytes in tissue culture [See T. DeFrance et al., J. Exp. Med. (1987), Vol. 165, 1459-1457]; (iii) IL-4 interacted in an extremely precise way with other lymphokines, notably interferon-γ [See R.L. Coffman et al. Immunol. Res. (1988), Vol. 102, 5-27 and Pene et al., supra] and T cells [See R.L. Coffman et al. supra, Pene et al. supra, and M.D. Widmer et al., Nature, (1987), Vol. 326, 795-98] to bring about B cell proliferation and alteration; and (iv) IL-4 increased MHC class II antigen expression on resting B cells (R Noelle et al., PNAS 81. 6149-6153, 1984). T.R. Mosmann et al. in J. Immuno., Vol. 138, 1813-1816 disclosed that human and murine IL-4 which are 50% homologous at amino acid sequence 1-90 and 129-149 were species specific.

Studies in humans showed that IL-4 has an effect on monoclonal B cell tumors. S. Karray et al. in J. Exp. Med. (1988), Vol. 168, 85-94, disclose that human IL-4 suppresses the IL-2-dependent proliferation of B-type chronic lymphocytic leukemia (B-CLL) in vitro. T. DeFrance et al. J. Exp. Med. (1988), Vol 168, 1321 disclose that IL-4 inhibits the in vitro proliferation but not the differentiation of activated human cells in response to IL-2. See also D.F. Jelinek et al. J. Immunol. (1988), Vol 141; 164. C.M. Higuchi et al. in

Can. Res. (1989), Vol. 49, 6487-6492, disclose that in human peripheral blood lymphocytes preactivated by IL-2, IL-4 induces lymphokine-activated killer activity (LAK). J.J. Mule et al. in J. Exp. Med. (1987), Vol. 166; 792-797, disclose that in the murine system, resting splenocytes treated with murine IL-4 alone or in combination with IL-2 generate LAK activity against fresh syngenic tumor cells in vitro. G. Forni et al. in Int. J. Can. Sup. (1989), Vol. 4; 62-65, disclose that antitumor activity can be induced by injecting murine IL-4 around the tumor draining lymph node and that when IL-4 is used in combination with a nonapeptide from human IL-1ß, very active lymphokine-activated tumor inhibition (LATI) is observed. J.J. Mule et al. in J. Immuno. (1989), Vol. 142; 726-733 disclose that the major phenotype of the cells induced by murine IL-4 is surface expression of asialo-GM_1, Thy^+, Lyt^{2+}, T^{3+} and that in LAK cells generated by a combination of IL-2 plus IL-4, there is an increase in granule-associated serine esterase. D.J. Peace et al. in J. Immuno. (1988), Vol. 140, 3679-3685 disclose that IL-4 induced LAK activity is associated with two different cell types, one NK-like ($NK1.1^+$, Lyt^{2-}) and the other T cells like ($NK\ 1.1^-$, Lyt^{2+}). R.I. Tepper et al. in Cell (1989), Vol. 57; 503-512 disclose that murine tumor cell lines transfected with murine IL-4 are inhibited from growing in vivo but that IL-4 transfected tumor cells mixed with nontransfected tumor cells resulted in the inhibition of the nontransfected tumor growth in vivo when the two tumor cell types were colocalized. R.I. Tepper et al. also disclose that when the nontransfected tumor was at a distal site from the IL-4-transfected tumor, inhibition was not observed. R.I. Tepper further disclose that parenteral administration of a cytokine, e.g. IL-2 or IL-4 to a tumor-bearing animal is "compromised by the short half life of the factor (as is the case for IL-2) and the need to obtain the cytokine in quantities sufficient to achieve effective dose

levels. G. D'Orazi et al. in Proceedings of the American Association for Cancer Research, (March 1990), Vol. 31, p. 252. Abstract N° 1490 disclose that IL-4 induced an antitumor response in a nude mouse model.

A method of treating solid tumors in mammals afflicted with solid tumors by systemically administering IL-4 to said mammals is disclosed in commonly-assigned US Patent Application Serial N° 07/496,832, filed March 21, 1990.

SUMMARY OF THE INVENTION

Surprisingly, we have found that a broad array of B-cell malignancies may be treated and the growth thereof inhibited by administering an effective amount of IL-4 into mammals, such as human beings, afflicted with such B-cell malignancies.

Accordingly, the present invention provides a pharmaceutical composition for treating a B-cell malignancy in a mammal afflicted with such a malignancy wherein said composition comprises IL-4 as an active ingredient.

The present invention also provides a pharmaceutical composition for inhibiting the growth of a B-cell malignancy in a mammal afflicted with a B-cell malignancy, wherein said composition comprises IL-4 as an active ingredient.

The present invention still further provides a pharmaceutical composition for inhibiting the proliferation of malignant B-cells in mammals afflicted with malignant B-cells, wherein said composition comprises IL-4, as an active ingredient.

Furthermore, the present invention provides a method of treating a B-cell malignancy in a mammal afflicted with

a B-cell malignancy which comprises administering to said mammal an amount of IL-4 effective for such treating.

The present invention also provides a method of inhibiting the growth of a B-cell malignancy in a mammal afflicted with a B-cell malignancy which comprises administering to said mammal an amount of IL-4 effective for such inhibiting.

The present invention still further provides a method of inhibiting the proliferation of malignant B-cells in mammals afflicted with malignant B-cells which comprises administering to said mammals an amount of IL-4 effective for such inhibiting.

And the present invention has also for its object a use of IL-4 for the manufacture of a medicament for treating a B-cell malignancy in a mammal afflicted with such a malignancy.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the dose-response curve of the growth-inhibitory effect in vitro of IL-4 in accordance with this invention on the IL-2 driven proliferation of, malignant B-cells from lymph nodes.

Figure 2 illustrates the dose-response curve of the growth-inhibitory effect of IL-4 in accordance with this invention on the anti-IgM-induced proliferation of, malignant B-cells from lymph nodes.

DETAILED DESCRIPTION OF THE INVENTION AND OF THE PREFERRED EMBODIMENTS

The term "B-cell malignancy" and the term "malignant B-cell" as used herein refer to a broad array of B-cell

tumors and B-cell neoplasms of a low, intermediate or mature grade of malignancy and include but are not limited to leukemic B-cells such as chronic lymphocytic leukemias (hereinafter "CLL") B cells, B-cell lymphomas including non-Hodgkin malignant lymphoma (hereinafter "NHML") B-cells, centroblastic-centrocytic lymphoma, follicular small-cleaved cell ("FSC") B-cell lymphoma, diffuse small-cleaved cell ("DSC") B-cell lymphoma, small non-cleaved cell ("SNC") B-cell lymphoma, immunoblastic large cell ("IBS") B-cell lymphoma, small cell lymphocytic ("SCL") B-cell lymphoma and diffuse large cell ("DLC") B-cell lymphoma as well as B-cell lymphomas disclosed by R.A. Miller et al. NEJM (1989), Vol 321; 851-856.

In a pre-clinical study done in vitro, we have found that IL-4 administered in accordance with this invention inhibited interleukin-2 ("IL-2") induced proliferation of malignant B-cells in fifteen (15) of sixteen (16) patients with chronic lymphocytic leukemia ("CLL") and in seven (7) of ten (10) patients with low grade lymphomas. In one clinical study, 5 microgram/kg/day of IL-4 was administered by bolus subcutaneous injection to ten patients including one patient with CLL and one patient with a low grade lymphoma. There was a rapid shrinkage of lymph nodes and spleen in the low grade lymphoma patient and more than three fold fall in the circulating malignant B-cells with six (6) hours of a single 5 microgram/kg subcutaneous injection of IL-4 in the CLL patient.

In an in vitro study, we demonstrated that IL-4 inhibited the in vitro proliferative response of freshly-isolated non-Hodgkin malignant lymphoma (hereinafter "NHML") B-cells. For this in vitro antiproliferation assay, the leukemic NHML B cells were activated with insolubilized anti-IgM antibodies or Staphylococcus Aureus Strain Cowan 1 (hereinafter

"SAC"). For 8 of the 9 leukemic NHML B cells, IL-2 was the sole cytokin/B cell tropic factor which significantly and reproducibly stimulated DNA synthesis in these NHML B-cells activated through their surface Igs. IL-4 strongly suppressed the proliferative signals delivered to the NHML B cells either by anti-Ig reagents alone or by the combination of IL-2 and anti-Ig reagents. These in vitro data suggest that IL-4 essentially provides growth inhibitory signals to NHML B-cells where said B-cells are activated through their surface Ig receptors. It is expected based on these in vitro results that IL-4 would be useful in clinical treatment of mature B-cell malignancies.

The phrase "treating a B-cell malignancy" as used herein means a broad range of anti-B cell responses resulting from administration of IL-4 in accordance with this invention including: (1) effecting B-cell malignancy regression; (2) inhibiting malignant B-cell growth; (3) inhibiting the proliferation of malignant B-cells; (4) inducing an effective immune response to inhibit malignant B-cell growth or effect malignant B-cell regression; and (5) augmenting an effective immune response to effect inhibition or regression of malignant B-cell growth in mammals. The immune response of some mammals in the absence of IL-4 administered in accordance with this invention may not be strong enough or fast enough to effect malignant B-cell growth inhibition or malignant B-cell regression but we have found effective methods which comprise administering to malignant B-cell bearing mammals an amount of IL-4, preferably recombinant IL-4, effective for each of such purposes.

Any suitable IL-4 may be employed in the present invention. Complementary DNAs (cDNAs) for IL-4 have recently been cloned and sequenced by a number of laboratories, e.g. Yokota et al., Proc. Natl. Acad. Sci.

USA. (1986), Vol. 83; 5894-5898 (human); Lee et al., Proc. Natl. Acad. Sci. USA (1986), Vol. 83; 2061-2065 (mouse); Noma et al., Nature (1986), Vol. 319; 640-646 (mouse); and Genzyme Corporation, Boston, Massachusetts (human and mouse). Moreover, non-recombinant IL-4 has been purified from various culture supernatants, e.g.; Grabstein et al., J. Exp. Med. (1985), Vol. 163; 1405-1413 (mouse); and Ohara et al., J. Immunol. (1985), Vol. 135; 2518-2523 (mouse BSF-1). The disclosure of all the above articles are incorporated herein by reference for their teachings of DNA and amino acid sequences and of methods of obtaining suitable IL-4 materials for use in the present invention.

Preferably, the IL-4 used in the present invention is human IL-4, and most preferably it is the human version with the sequence described in Yokota et al., Proc. Natl. Acad. Sci. USA (1986), Vol. 83; 5894-5898 and PCT Patent Application N° 87/02990 published May 21, 1987 that is expressed in and isolated from E. Coli (U.S. Patent Application N° 079,666, filed July 29, 1987 and U.S. Patent Application N° 194,799, filed July 12, 1988). The production of IL-4 from CHO cells is described in commonly-owned U.S. Patent Application SN 386,937, filed July 28, 1989. The production of IL-4 from E. coli is described in commonly-owned U.S. Patent Application SN 429,588, filed October 31, 1989. The disclosures of the above article, PCT application and U.S. Patent Applications are hereby incorporated herein by reference.

According to this invention, mammals are administered an effective amount of an IL-4 to inhibit malignant B-cell growth, to effect malignant B-cell regression, to induce an effective immune response, to inhibit malignant B-cell growth or to effect malignant B-cell regression or to augment an effective immune response to effect solid tumor growth inhibition or solid tumor regression.

From about 0.25 to about 15 micrograms of IL-4, preferably human IL-4 ("hIL-4") recombinantly produced from E. coli or CHO cells, more preferably E. coli-derived recombinant hIL-4, per kilogram of body weight per day is preferably administered. More preferably, mammals are administered about 5 to about 15 micrograms of recombinant hIL-4 per kilogram of body weight per day, and most preferably mammals are administered about 5 to about 10 micrograms of recombinant hIL-4 per kilogram of body weight per day in single or divided doses.

The amount, frequency and period of administration will vary depending upon factors such as the level of the neutrophil and monocyte count (e.g., the severity of the monocytopenia or granulocytopenia), age of the patient, nutrition, etc. Usually, the administration of IL-4 will be daily initially and it may continue periodically during the patient's lifetime. Dosage amount and frequency may be determined during initial screenings of neutrophil count and the magnitude of the effect of IL-4 upon the increase in antibody levels.

Administration of the dose can be intravenous, parenteral, subcutaneous, intramuscular, or any other acceptable systemic method. The IL-4 can be administered in any number of conventional dosage forms. Parenteral preparations include sterile solutions or suspensions. Dosages of more than about 10 to about 15 micrograms of recombinant IL-4 per kilogram of body weight are preferably intravenously or subcutaneously (e.g. by bolus s.c.) administered to human beings.

The formulations of pharmaceutical compositions contemplated by the above dosage forms can be prepared with conventional pharmaceutically acceptable excipients and additives, using conventional techniques.

Presently, the IL-4 is preferably administered systemically via injection, preferably via subcutaneous bolus or intraperitoneal injection or even intravenous injection. The solutions to be administered may be reconstituted lyophilized powders and they may additionally contain preservatives, buffers, dispersants, etc.

Preferably, IL-4 is reconstituted with 10 millimolar citrate buffer and preservative-free sterile water with the maximum concentration not to exceed 100 micrograms per milliliter and administered systemically via subcutaneous injection, intraperitoneal injection or via continuous intravenous infusion or by intravenous injection. For continuous infusion, the daily dose can be added to 5 ml of normal saline and the solution infused by mechanical pump or by gravity.

The effect of IL-4 on B-cell malignancies can be determined inter alia by shrinkage of lymph nodes and spleen, a fall in the circulatory malignant B-cells as well as by the reduction in tumor volume (which can be measured using standard techniques such as caliper measurements, X-ray and MRI) as well as by increased life span or survival of such mammals by the following test protocol.

MATERIALS AND METHODS

Isolation and purification of malignant (leukemic) B cells

Pathological samples were provided by Pr. Sotto (Hospital Albert Michallon, Grenoble, France), Dr. J.F. Rossi (Hospital Val d'Aurelle, Montpellier, France) and Dr. J.P. Magaud (Hospital Edouard Herriot, Lyon, France). Nine patients with the diagnosis of low grade non Hodgkin, non Burkitt, malignant lymphoma (NHML)

B-cells according to the Kiel classification, who have not received chemotherapy for the last four months preceding surgery were selected for this study. The available specimens included 5 lymph nodes (EMZ, GAN, PP, MAI, PRO) and 4 spleens (BOU, DEL, BRE, THE). Mononuclear cells were obtained after dilaceration of the organs on a steel mesh and centrifugation of the cell suspension over Ficoll/Hypaque gradient. For B-cell purification, mononuclear cells were first submitted to E rosetting with sheep red blood cells. The non-rosetting cells (E^- fraction) were subsequently incubated with a cocktail of anti-T cells (anti-CD3, anti-CD2) and anti-monocytes (anti-CD14) monoclonal antibodies. Residual non-B cells were next removed from the E^- population after incubation with magnetic beads (Dynabeads, Dynal, Oslo, Norway) coated with anti-mouse IgG.

Reagents

Insolubilized anti-IgM antibodies were purchased from BioRad Laboratories (Richmond, CA) and were used at the final concentration of 10 µg/mL. Formalinized particles of Staphylococcus Aureus Strain Cowan I (SAC) were purchased as Pansorbin from Calbiochem-Behring Corporation (La Jolla, CA). SAC was used at 0.005% final concentration (w/v).

Cytokines

Purified recombinant IL-2 (3×10^6 U/ml) was purchased from Amgen Biologicals (Thousand Oaks, CA) and used at the final concentration of 20 U/mL which was determined to be optimal for the growth of normal B cells co-stimulated with insolubilized anti-IgM antibodies. Purified recombinant human IL-4 (derived from E. coli, 1×10^7 U/mg) was provided by Drs. P. Trotta and T.L. Nagabhushan (Schering-Plough Research, Bloomfield, NJ).

In the experiments, IL-4 was used at the final concentration of 500 U/ml which concentration provides maximal stimulation of B cell growth, as estimated on normal B cells activated with insolubilized anti-IgM antibodies.

Cultures

The nine purified leukemic NHML B cells were cultured in Iscove's medium (Flow Laboratories, Irvine, CA) enriched with 50 µg/mL of human transferrin, (Sigma Chemical Co., St Louis, MO), 0.5% bovine serum albumin (Sigma), 5 µg/mL of bovine insulin (Sigma), 5% selected heat inactivated fetal calf serum, 100 U/ml of penicillin, 100 µg/ml of Streptomycin (all from Flow Laboratories) and 10^{-5}M of ß mercaptoethanol (Sigma). For proliferation assays, 1 x 10^5 of leukemic B-cells were plated in 100 µL of culture medium in round-bottom microwells and incubated in a 5% CO_2 humidified atmosphere at 37°C for five days in the presence of Polyclonal B cell activators PBA-SAC, or insolubilized anti-IgM antibodies and/or factors IL-4 (500 U/ml) and/or IL-2 (20 U/mL) which were added at the onset of the culture. DNA synthesis was determined by pulsing cells with (3H)thymidine (hereinafter "(3H)TdR") for the final 16h of the culture period. Due to the heterogeneity of the responses of leukemic B cell samples, DNA synthesis was assessed at four different time intervals (on days 3, 4, 5 and 6) after onset of the culture. The results presented in Table I correspond to the time point which provided the maximal stimulation indices. Counts per minute of (3H)TdR incorporation (cpm $X10^{-3}$) are expressed as means of triplicate determinations. Standard deviation never exceeded 10% of the mean value. The results are listed in Table I.

IL-4 was assayed for its capacity to antagonize the proliferative response of NHML B-cells to IL-2. Each of

the 9 specimens of NHML B-cells was therefore co-cultured with IL-2 (20 U/ml) and IL-4 (500 U/ml) in the presence of the anti-Ig reagent previously defined as being optimal for activation. Since the leukemic samples also differed from one to another in terms of time kinetics of the response to the growth-promoting effect of IL-2, DNA synthesis was measured 4, 5, 6 and 7 days after onset of the culture. Data displayed in Table I represent, for each clone, the levels of (3H)TdR incorporation obtained at the time point corresponding to the peak of the proliferative response to IL-2. One clone (MAI) did not display significant growth response to IL-2 or IL-4 in both activation systems and therefore is not included in Table I.

In 7 out 8 cases, IL-4 failed to synergize with anti-Ig reagents to support DNA synthesis from NHML B-cells. However, one clone (BRE) exhibited the opposite pattern of response and proliferated upon culturing with IL-4 and anti-IgM antibodies. With the exception of clone BRE, all NHML B-cells samples displayed a growth response to IL-2. For all IL-2 responsive clones, IL-4 was found to significantly inhibit the IL-2 driven proliferation of the cells. The titration of the growth-inhibitory effect of IL-4 on the IL-2 induced response of 1×10^5 cells of the leukemic clone GAN co-stimulated with 0.005% of SAC and 10 U/mL of IL-2 in the absence or in the presence of serial dilutions of IL-4. The incorporation of (3H)TdR was assessed on day 4. Figure 1 is dose-response curve which is representative of three experiments and graphically shows that complete inhibition of the response to IL-2 is already achieved for concentrations of IL-4 as low as 16 U/mL.

Figure 2 is a dose response curve which graphically summarizes four experiments and shows the growth inhibiting effect of IL-4 on the anti-Ig M-induced

proliferation of clone PRO. These leukemic B-cells (1×10^5 of PRO) were stimulated with insolubilized anti-Ig M antibodies (10 µg/mL), in the absence or in the presence of serial dilutions of IL-4. The (3H)TdR was added during the last 16h of a 4 day culture period. The (3H)TdR incorporation is unstimulated cultures was 346 ± 28 cpm. Moreover, IL-4 also inhibited the growth of the three other NHML B-cells specimens (BOU, EMZ, PRO) which already exhibited a significant proliferative response upon ligation of their surface Igs with SAC or anti-IgM antibodies (Table I, Fig. 2). In the present study, we show that, in their great majority, NHML B-cells activated through their surface Ig receptors can be stimulated for DNA synthesis by IL-2. In some tumor specimens (3 out of 9), a proliferative response could also be induced by the sole ligation of the surface Igs in the absence of exogenous growth factors. IL-4 significantly suppressed not only the growth response of these cells to IL-2 but also to the anti-Ig reagent by itself.

TABLE I

ANTI-PROLIFERATIVE EFFECTS OF IL-4 ON IL-2 INDUCED GROWTH OF FRESHLY ISOLATED NON HODGKIN MALIGNANT LYMPHOMA ("NHML") B CELLS[1]

	PBA=	SAC[2]			PBA=		Anti-IgM	
NHML B cells[3]:	BOU	THE	PP	DEL	GAN	EMZ	PRO	BRE
Medium	0.5	0.3	0.2	1.2	0.6	0.2	0.3	0.2
PBA[4]	21.4	2.3	6.2	0.6	0.6	18.2	64.8	2.4
PBA+IL-4[5]	11.9	2.0	3.0	1.6	4.0	7.8	30.1	9.1
PBA+IL-2[6]	35.5	7.9	47.2	8.4	22.2	56.8	72.3	4.5
PBA+IL-2+IL-4	12.8	3.9	9.5	3.7	9.5	13.6	27.8	11.1

1. (3H)TdR incorporation (cpm x 10^{-3}) measured after five days and expressed as the mean of triplicate determinations.
2. SAC = Staphylococcus Aureus Strain Cowan 1.
3. 1 x 10^{5} of NHML cells incubated at 37° for 5 days.
4. PBA = Polyclonal B cell Activator.
5. 500 U/mL of IL-4
6. 20 U/mL of IL-2

CLAIMS

1. A pharmaceutical composition for treating a B-cell malignancy in a mammal afflicted with a B-cell malignancy wherein said composition comprises an effective amount of IL-4 as an active ingredient.

2. The composition according to claim 1 for inhibiting the growth of a B-cell malignancy in a mammal afflicted with a B-cell malignancy.

3. The composition according to claim 1 for inhibiting the proliferation of malignant B-cell in mammals afflicted with malignant B-cells.

4. The composition according to claim 1, 2 or 3, in a form adapted to be intravenously or intraperitonealy administered.

5. The composition according to claim 1, 2 or 3, in a form adapted to be subcutaneously administered.

6. The composition according to claim 1, 2 or 3, wherein said active ingredient is human IL-4.

7. The composition according to claim 1, 2 or 3, wherein said active ingredient is E. Coli-derived recombinant human IL-4.

8. The composition according to claim 1, 2 or 3, wherein said effective amount of IL-4 is in the range of about 0.25 to about 15 micrograms per kilogram of body weight for a daily administration.

9. A method of treating a B-cell malignancy in a mammal afflicted with such a malignancy which comprises administering an amount of IL-4 effective for such treating.

10. A method of claim 9, wherein the treating comprises inhibiting the growth of a B-cell malignancy in a mammal afflicted with a B-cell malignancy by administering to said mammal an amount of IL-4 effective for such inhibiting.

11. A method of claim 9, wherein the treating comprises inhibiting the proliferation of malignant B-cells in mammals afflicted with malignant B-cells by administering to said mammal an amount of IL-4 effective for such inhibiting.

12. The method of any of claims 9, 10 or 11, wherein the IL-4 is administered intravenously.

13. The method of any of claims 9, 10 or 11, wherein human IL-4 is administered.

14. The method of any of claims 9, 10 or 11, wherein E. coli-derived recombinant human IL-4 is administered.

15. The method of any of claims 9, 10 or 11 wherein the IL-4 is administered subcutaneously.

16. The method of any of claims 9, 10 or 11, wherein the amount of IL-4 administered is in the range of about 0.25 to about 15 micrograms per kilogram of body weight per day.

17. Use of IL-4 in the manufacture of a medicament for treating a B-cell malignancy in a mammal afflicted with such a malignancy.

18. Use of IL-4 in the manufacture of a medicament for inhibiting the growth of a B-cell malignancy in a mammal afflicted with a B-cell malignancy.

19. Use of IL-4 in the manufacture of a medicament for inhibiting the proliferation of malignant B-cells in mammals afflicted with malignant B-cells.

20. The use according to claim 17, 18 or 19, wherein said medicament is in the form adapted to be intravenously or intraperitonealy administered.

21. The use according to claim 17, 18 or 19, wherein said medicament is in the form adapted to be subcutaneously administered.

22. The use according to claim 17, 18 or 19, wherein IL-4 is human IL-4.

23. The use according to claim 17, 18 or 19, wherein IL-4 is E. Coli-derived recombinant human IL-4.

24. The use according to claim 17, 18 or 19, wherein said medicament comprises an amount of IL-4 in the range of about 0.25 to about 15 micrograms per kilogram of body weight for a daily administration.

Fig. 1

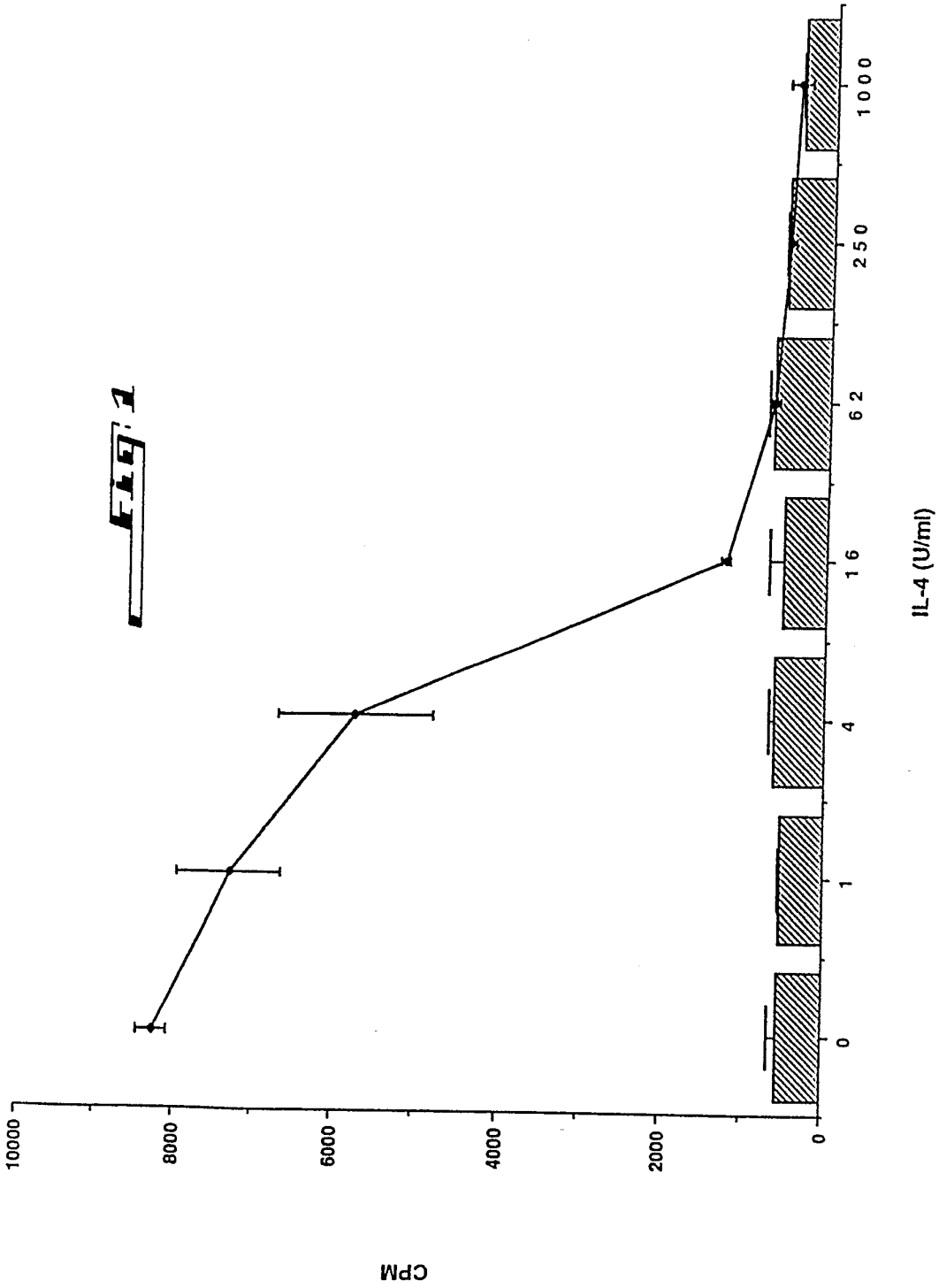
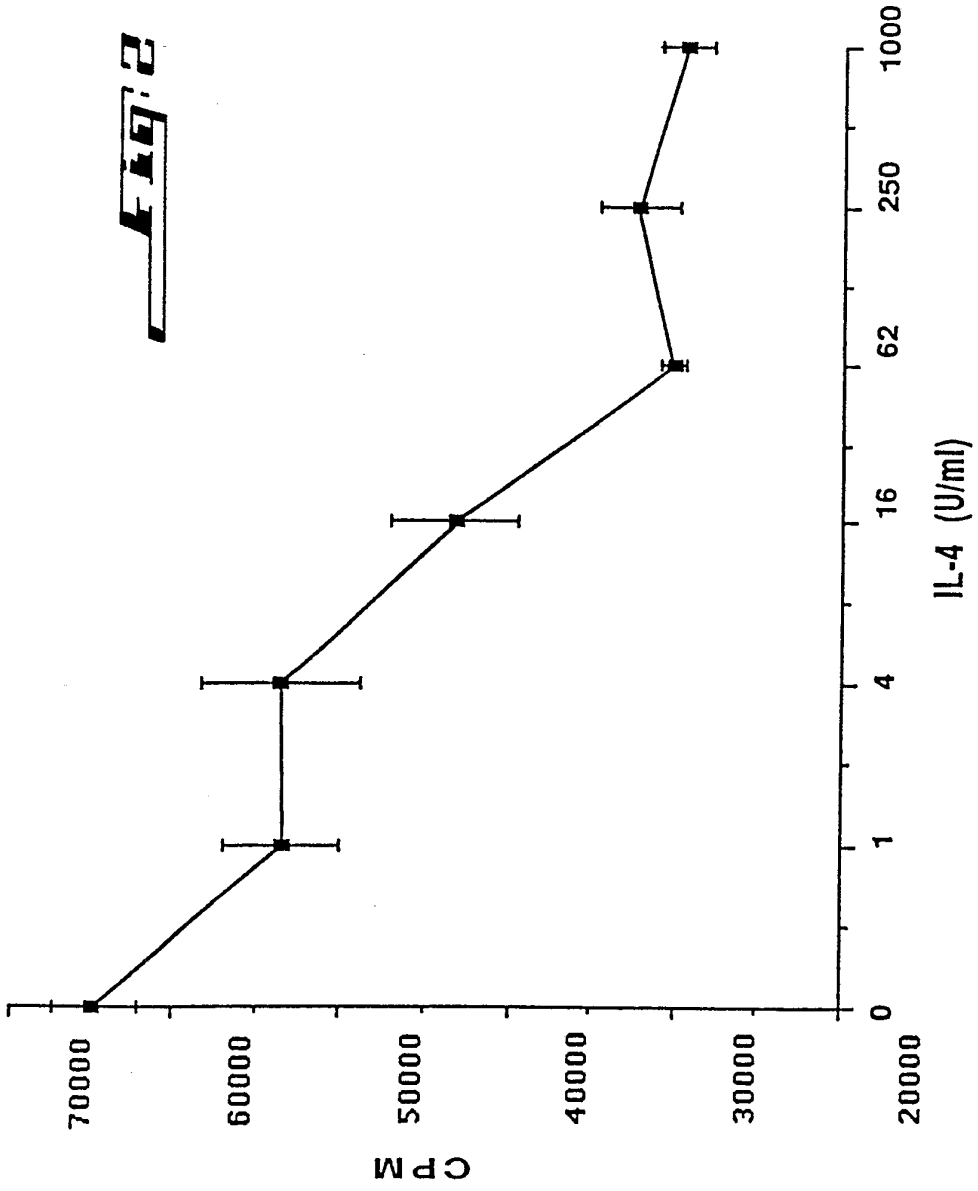


Fig. 2


CPM
70000
60000
50000
40000
30000
20000
0
1
4
16
62
250
1000
IL-4 (U/ml)

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 91/02380

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	Blood, volume 75, no. 5, 1 March 1990, Saunders Comp., (Duluth, US) C.W. Taylor et al.: "Effects of interleukin-4 on the in vitro growth of human lymphoid and plasma cel neoplasms", pages 1114-1118, see especially page 1117, table 3 and left-hand column, line 3 - right-hand column, line 13 ---	1-24
X	The Journal of Experimental Medicine, volume 168, no. 1, 1 July 1988, Rockefeller University Press (New York, US) S. Karray et al.: "Interleukin 4 counteracts the interleukin 2-induced proliferation of monoclonal B cells", pages 85-94, see pages 85-94 (cited in the application) -----	1-24

* Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28-02-1992	18. 03. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Mme. M. van der Drift

FURTHER INFORMATIOI ONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim numbers 9-16 because they relate to subject matter not required to be searched by this Authority, namely:

"REMARK : Although claims 9-16 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition."

2. [ ] Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.