PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 93/14782
A61K 37/02, 39/395	A1	(43) International Publication Date: 5 August 1993 (05.08.93)

(21) International Application Number: PCT/US93/00998

(22) International Filing Date: 28 January 1993 (28.01.93)

(30) Priority data:
827,106 29 January 1992 (29.01.92) US

(71) Applicant: LA JOLLA CANCER RESEARCH FOUNDATION [US/US]; 10901 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors: MAKI, Richard, A. ; 4175-174 Porte De Palmas, San Diego, CA 92122 (US). CELADA, Antonio ; Galileo 1, 70, 1, E-08028 Barcelona (ES).

(74) Agents: KONSKI, Antoinette, F. et al.; Campbell & Flores, 4370 La Jolla Village Drive, Suite 700, San Diego, CA 92122 (US).

(81) Designated States: AU, BB, BG, BR, CA, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS OF CONTROLLING THE PROLIFERATION OF MACROPHAGES

(57) Abstract

This invention provides a method for stimulating or inhibiting the proliferation of differentiated macrophages by modulating the amount or activity of TGF-β in contact with said differentiated macrophages, as by contact with an effective amount of M-CSF or GM-CSF and TGF-β. The invention further provides a method for treating an individual with a condition characterized by the overabundance or lack of adequate differentiated macrophages by modulating the amount or activity of TGF-β activity in macrophage-containing tissues of said individual, as by administering an effective amount of M-CSF or GM-CSF and TGF-β, or by contacting differentiated macrophages with an agent, such as an antibody or decorin, which suppresses the activity of TGF-β. Further, the invention provides methods for stimulating proliferation of immature macrophages by contact with TGF-β.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**MR**	Mauritania
AU	Australia	**GA**	Gabon	**MW**	Malawi
BB	Barbados	**GB**	United Kingdom	**NL**	Netherlands
BE	Belgium	**GN**	Guinea	**NO**	Norway
BF	Burkina Faso	**GR**	Greece	**NZ**	New Zealand
BG	Bulgaria	**HU**	Hungary	**PL**	Poland
BJ	Benin	**IE**	Ireland	**PT**	Portugal
BR	Brazil	**IT**	Italy	**RO**	Romania
CA	Canada	**JP**	Japan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SK**	Slovak Republic
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SN**	Senegal
CM	Cameroon	**LI**	Liechtenstein	**SU**	Soviet Union
CS	Czechoslovakia	**LK**	Sri Lanka	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**MC**	Monaco	**UA**	Ukraine
DK	Denmark	**MG**	Madagascar	**US**	United States of America
ES	Spain	**ML**	Mali	**VN**	Viet Nam
FI	Finland	**MN**	Mongolia		

METHODS OF CONTROLLING THE PROLIFERATION OF MACROPHAGES

The United States Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of Grant No. A120194 awarded by the United States Public Health Service.

BACKGROUND OF THE INVENTION

The present invention generally relates to controlling the proliferation of macrophages, and more particularly to the stimulation or inhibition of macrophage proliferation by modulating the amount or activity of TGF-ß in contact with such macrophages.

Macrophages originate from stem cells in the bone marrow which, after differentiation to blood monocytes, settle in various tissues as mature or differentiated macrophages. The grouping of such differentiated macrophages make up the mononuclear phagocyte system. Macrophages are present throughout the connective tissue and around the basement membrane of small blood vessels and concentrate in various areas, for example, in the lung (alveolar macrophages), liver (Kupffer cells), the lining of spleen sinusoids and lymph node medullary sinuses to filter off foreign material. Other tissues in which macrophages accumulate include mesangial cells in the kidney glomerulus, brain microglia and osteoclasts in bone.

Mature macrophages are known to combat various microbes, including bacteria, viruses and protozoa, and particularly those capable of living within the cells of the host. The mononuclear phagocyte system operates as a defensive mechanism to rid such foreign microbes as well as other foreign particles. Macrophages contain lysosomes and an oxidative microbicidal system that degrade the foreign material.

Macrophages are also important accessory cells for presentation of antigens to T-cells. Antigens ingested by macrophages do not become wholly degraded. As a result, peptide fragments of such antigens appear on the cell surface, where they can be recognized by antigen receptors of T cells. This recognition further triggers the immune response, including the endogenous production of antibodies against the antigen.

Controlling the proliferation of macrophages would therefore be useful in the prevention, suppression or treatment of various pathologies, including for example, infections caused by invasion of foreign microbes or conditions relating to cancer-causing or other disease-related antigens. For instance, stimulating macrophage proliferation would be particularly useful in the case of immunocompromised individuals. In addition, the rejection of grafts may be reduced by inhibiting macrophage proliferation.

Thus, a need exists for methods that can stimulate or inhibit the proliferation of macrophages depending on the desired immune response for a variety of different circumstances. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

This invention provides a method for stimulating or inhibiting the proliferation of differentiated macrophages by modulating the amount or activity of TGF-ß in contact with said differentiated macrophages, as by contact with an effective amount of M-CSF or GM-CSF and TGF-ß. The invention further provides a method for treating an individual with a condition characterized by the overabundance or lack of adequate differentiated macrophages by modulating the amount or activity of TGF-ß

activity in macrophage-containing tissues of said individual, as by contact with an effective amount of M-CSF or GM-CSF and TGF-ß, or by contacting differentiated macrophages with an agent, such as an antibody or decorin, which suppresses the activity of TGF-ß. Further, the invention provides methods for stimulating proliferation of immature macrophages by contact with TGF-ß.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows that TGF-ß enhances bone marrow-derived macrophages M-CSF dependent proliferation.

Figure 2 shows the different effects of TGF-ß on immature vs. committed macrophages.

Figure 3 shows the differential effect of TGF-ß on GM-CSF or IL-3-dependent proliferation.

Figure 4 shows that autocrine production of TGF-ß contributes to M-CSF or GM-CSF dependent proliferation.

DETAILED DESCRIPTION OF THE INVENTION

The present invention generally relates to controlling the proliferation of macrophages in various stages of differentiation. More specifically, macrophage proliferation is stimulated or inhibited by modulating the amount or activity of TGF-ß in contact with the targeted macrophages.

As used in reference to differentiated macrophages herein, the terms "committed" and "differentiated" are used interchangeably to mean mature macrophages. Such mature macrophages generally reside in tissues upon differentiating and migrating from the bone marrow, for example, peritoneal macrophages.

"Immature" macrophages are not differentiated and usually do not migrate out of the bone marrow. Nonadherent bone marrow derived macrophages ("BMM") are an example of a type of immature macrophage.

The term "modulating" refers to adjusting or adapting the amount or activity of TGF-ß to a certain proportion to achieve either inhibition or stimulation of macrophages, depending on the intended use.

In both bone marrow and tissues, macrophages proliferate in response to specific growth factors called colony stimulating factors ("CSF"), which are described in Metcalf, Blood 67:257 (1986). The autocrine production of growth factors has been suggested as one of the mechanisms responsible for the unregulated growth of hematopoietic cells. Macrophages produce a number of growth mediators that have a positive effect on proliferation, such as macrophage colony stimulating factor ("M-CSF"), granulocyte/macrophage colony stimulating factor ("GM-CSF") and interleukin-3 ("IL-3") and produce other factors, such as interferon α, that inhibit proliferation. Some factors also stimulate the production of other factors. For instance, M-CSF stimulates the production of tumor necrosis factor, which in turn, stimulates the expression of M-CSF.

Transforming growth factor ß ("TGF-ß") is a polypeptide which is expressed in various tissues and has the unusual ability to both stimulate and inhibit the proliferation of various cells in culture as described in Massage, Annual Rev. Cell Biol., 6:597 (1990) and Moses et al., Cell 63:245 (1990). This factor is produced by macrophages, induces macrophage chemotaxis, and modulates the production of cytokines such as IL-1 and tumor necrosis factor. Finally, TGF-ß is also able to upregulate its own expression in macrophages.

The results of various studies relating to the effects of TGF-ß on hematopoietic cell growth and differentiation indicate that TGF-ß inhibits hematopoietic progenitor cell growth. In particular, TGF-ß was shown to be a selective inhibitor of colony-stimulating factor-driven growth of both murine and human hematopoietic progenitor cells as described in Ohta et al., Nature 329:539 (1987); Keller et al., J. Exp. Med. 168:737 (1988); Keller et al., Blood 75:596 (1990); and Cashman et al., Blood 75:96 (1990). TGF-ß was also shown to inhibit the colony growth of multipotential hematopoietic progenitors, while more committed progenitors were insensitive as reported in Agiletta et al., Exp. Hematol. 17:296 (1989); Ottmann and Pelus, J. Immunol. 140:2661 (1988); and Lotem and Sachs, Blood 76:1315 (1990).

In another study using the method of soft agar colony formation, TGF-ß1 was shown to be a selective inhibitor of early hematopoiesis as reported in Ohta et al., supra; Keller et al., supra; and Sing et al., Blood 72:1504 (1988). Specifically, TGF-ß was found to be a potent inhibitor of IL-3-induced bone marrow proliferation and colony formation as reported in Keller et al., 1988 supra; Keller et al., 1990 supra; and Kishi et al., Leukemia 3:687 (1989). In other studies, TGF-ß was shown to have little or no effect on the proliferation and differentiation of early hematopoietic progenitors induced by G-CSF, M-CSF or erythropoietin. These studies are described in Keller et al., supra; Sing, supra; Keller et al., Blood 75:596 (1990); and Ottmann, supra. Finally, it has been shown that TGF-ß stimulated the growth of colony forming unit granulocyte/macrophage ("CFU-GM") as reported in Agiletta et al., supra and Ottmann et al., supra.

Despite the research in this area, there was little information on the role of TGF-ß on the proliferation of macrophages until the present invention. In studies relating to the present invention it was demonstrated that the response to TGF-ß depends not only on the state of cell maturation but also on the growth factor present in the assay. TGF-ß enhanced the proliferative activity of M-CSF and GM-CSF, but inhibited IL-3 induced proliferation. For committed macrophages, TGF-ß enhances both M-CSF and GM-CSF dependent proliferation. In contrast, for immature macrophages induced by a colony stimulating factor, TGF-ß inhibits proliferation. These results indicate that, depending on the degree of differentiation of macrophages, TGF-ß has a different effect on proliferative activity.

Although not wishing to be limited to any particular mechanism by which TGF-ß stimulates or inhibits cellular proliferation, the data obtained in this research suggests that it could involve a mechanism of interaction between specific growth factor pathways rather than a general effect on proliferation. It has been proposed that TGF-ß acts as an indirect mitogen on aortic smooth muscle cells by the autocrine production of platelet-derived growth factor ("PDGF") in Battegay et al., Cell 63:515 (1990). However, this proposal probably cannot explain the effect on macrophage proliferation because macrophage-derived PDGF is secreted in the presence of a binding protein that can inhibit its ability to bind to the PDGF cell surface receptor as reported in Skimokado et al., Cell 43:277 (1985). This mechanism also does not explain why TGF-ß enhances M-CSF and GM-CSF but not IL-3-induced proliferation in committed cells. Therefore, an alternative mechanism may exist to explain the enhancing effect of TGF-ß on macrophage proliferation.

It has been shown that TGF-ß blocks c-myc

expression at the transcriptional level. In keratinocytes c-myc expression is necessary for proliferation. It has been suggested that TGF-ß mediated growth inhibition involves the synthesis or modification of a protein that interacts with a cis-acting element in the 5′ regulatory region of c-myc, resulting in the inhibition of transcription of this gene. It is not known if this mechanism is responsible for the inhibition of proliferation of immature macrophages.

The present invention provides functional evidence of the autocrine production of TGF-ß, which then contributes to macrophage proliferation when the differentiated cells are treated with M-CSF or GM-CSF. It has been reported that activated macrophages are able to secrete TGF-ß and, in turn, TGF-ß is known to modulate the production of cytokines or various growth factors identified in Wahl et al, Proc. Natl. Acad. Sci. (USA), 84:5788 (1987); Chantry et al., J. Immunol. 142:4295 (1989); and McCartney-Francis et al., Growth Factors 4:27 (1990). In macrophages, TGF-ß has been shown to upregulate its own expression. Thus, TGF-ß has the ability to activate its own expression and thereby increase its own secretion. This auto-induction seems to be mediated by two distinct regions in the promoter of the TGF-ß gene and probably involves the binding of AP-1 (Jun-Fos) as described in Kim et al., Mol. Cell. Biol. 10:1492 (1990).

The results presented herein have therapeutic relevance. Immature macrophages grow in the bone marrow, where their proliferation could be controlled by various factors. Monocytes released from the bone marrow migrate to different tissues, where they differentiate into macrophages and presumably into other related cell types.

In tissues, under the effect of M-CSF or GM-CSF, macrophages then proliferate and probably respond to the

autocrine production of TGF-ß. An example of this latter situation may be found in the formation of granulomas which can be encountered in the course of certain inflammatory responses. Each granuloma could be viewed as a small spherical organ made of a variety of differentiated macrophages whose function is to limit the expansion of and allow the eventual destruction of intravascular bacteria.

It has been reported that TNF released from macrophages in the microenvironment of developing granulomas is involved in a process of autoamplification. TNF and TGF-ß acting in an autocrine or paracrine manner may favor further macrophage accumulation and differentiation in granulomas leading to bacterial elimination. Therefore, depending on the localization and degree of maturation, the production of TGF-ß by macrophages could enhance or decrease macrophage production, thereby contributing to a normal immune response.

The present invention accordingly provides methods for stimulating or inhibiting the proliferation of differentiated macrophages, comprising modulating the amount or activity of TGF-ß in contact with said differentiated macrophages. In one embodiment, the methods are used to stimulate proliferation of differentiated macrophages induced by a colony stimulating factor ("CSF"), such as M-CSF or GM-CSF, and later contacted with an effective amount of TGF-ß.

The present invention further provides methods of inhibiting the proliferation of differentiated macrophages by contacting the macrophages with an agent that suppresses the activity of TGF-ß. In one embodiment, the agent can be an antibody having specific reactivity with TGF-ß such that the resulting complex prevents TGF-ß from influencing the growth of said macrophages. The antibodies can be

prepared by any means known in the art, including antiserum (i.e., polyclonal) production or hybridoma techniques for producing monoclonal antibodies.

Other TGF-ß suppressors include decorin or its functional equivalent. As used herein, "decorin" refers to a proteoglycan having substantially the structural characteristics attributed to it in Krusius and Ruoslahti, Proc. Nat'l Acad. Sci. (USA) 83:7683 (1986), incorporated herein by reference. Decorin, also known as PG-II or PG-40, is a small proteoglycan produced by fibroblasts. Its core protein has a molecular weight of about 40,000 daltons. The molecule is known to carry a single glycosaminoglycan chain of a chondroitin sulfate/dermatan sulfate type. The term "decorin" also refers both to the native composition and to modification thereof that substantially retain the functional characteristics of native decorin. Decorin can be rendered glycosaminoglycan-free by mutation or other means, such as by producing recombinant decorin in cells incapable of attaching glycosaminoglycan chains to a core protein.

Functional equivalents of decorin include modifications of decorin that retain its functional characteristics and molecules that are homologous to decorin, such as biglycan and fibromodulin, for example, the addition of one or more side chains that do not interfere with the functional activity of the decorin core protein.

Methods for treating an individual with a condition characterized by the underexpression or overexpression of differentiated macrophages are also provided. Such methods are accomplished by modulating the amount or activity of TGF-ß in tissues containing the differentiated macrophages. For example, an effective amount of the TGFß and a colony stimulating factor ("CSF")

are administered to a patient to stimulate the proliferation of said macrophages. The CSF can be, for example, M-CSF or GM-CSF.

These methods are particularly useful for conditions characterized by the underexpression of macrophages, for example, when an individual is immunocompromised. Those skilled in the art can readily determine other uses of the claimed methods in which the stimulation of macrophages would be helpful. In addition, one skilled in the art can readily determine an effective amount of TGF-ß or CSF that can be used to stimulate the production of mature macrophages, as, for example, by monitoring the effects in a bone marrow sample.

In a further embodiment, the present invention relates to treatment methods useful for inhibiting the proliferation of macrophages using, for example, an agent that suppresses the activity of TGF-ß. These methods can be used prevent or treat conditions characterized by an overexpression of differentiated macrophages. Such agents include, for example, anti-TGF-ß antibodies, decorin and its functional equivalents. One skilled in the art can readily determine a therapeutically effective amount of such suppressive agents.

The present invention also provides methods for inhibiting or stimulating the proliferation of immature macrophages. For inhibiting the proliferation of such macrophages that have been induced to proliferate with a CSF, an effective amount of TGF-ß can be added to inhibit the proliferation. One skilled in the art would be able to determine an effective amount of TGF-ß without undue experimentation.

For stimulating the proliferation of immature macrophages not induced to proliferate with a CSF, an

effective amount of TGF-ß can be contacted with the macrophages. The effective amount of TGF-ß for use in these methods can also be readily determined by those skilled in the art without undue experimentation.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Preparation of Macrophages

Macrophages derived from bone marrow cultures ("BMM") were obtained as described in Celada et al., Eur. J. Immunol. 19:1103 (1989), incorporated herein by reference. Six week old DBA/2 mice (Jackson Labs., Bar Harbor, ME) were killed by cervical dislocation, and both femurs were dissected free of adherent tissue. The ends of the bones were cut off and the marrow tissue eluted by irrigation with PBS. The marrow plug was dispersed by passing through a 25 gauge needle and the cells were suspended by vigorous pipetting and washed by centrifugation. Cells (10^7) were cultured in plastic, tissue culture dishes (150 mm) in 50 ml of DME media containing 2 mM L-glutamine, 1 mM Na pyruvate, 50 U/ml penicillin, 50 µg/ml streptomycin, 20% FCS and 30% L cell-conditioned medium (DMEM supplemented with M-CSF (1200 U/ml) ("LCM"). The cell suspensions were incubated at 37°C in a humidified 5% CO_2 atmosphere. After 48 hours of incubation, nonadherent cells were collected and the adherent cells were discarded. The non-adherent population was cultured in plastic, nontissue culture (150 mm) dishes. After 7 days, macrophages were harvested with cold PBS.

Peritoneal elicited macrophages ("PEM") were prepared as described previously in Celada et al., J. Exp. Med. 160:55 (1984), incorporated herein by reference, by lavage from mice that had been injected i.p. 3 days

previously with 1.5 ml of 10% protease peptone (Difco Laboratories, Detroit, MI). Macrophage monolayers were prepared by seeding the PEM suspension into flat-bottom, 24-well tissue culture plates. The cells were allowed to adhere for 2 hours at 37°C before the plates were washed rigorously to remove nonadherent cells.

EXAMPLE II

Proliferation Assay

Cell proliferation was measured as previously described in R.I. Freshney, Culture of Animal Cells. A manual of Basic Technique 2 ed., p. 227 (1987), incorporated herein by reference, with minor modifications. Cells were incubated for 24 hours in 24 well plates (COSTAR, Cambridge, MA) in 1 ml of media with the indicated growth factor. Media was aspirated and replaced by 0.2 ml of media containing ^{3}H-thymidine (1.0 μCi/ml). After 2 hours of incubation at 37°C media was removed and cells were fixed in methanol. After 3 washes with 10% trichloroacetic acid, cells were solubilized in 1% SDS and 0.3 M NaOH. Radioactivity was counted by liquid scintillation. All samples were prepared in triplicate, and the results were expressed as the mean ± SD. In some experiments, cells were trypsinized and counted using a Coulter counter (ZM model, Hialeah, FL). Statistical analyses were performed using the Student's t-test.

EXAMPLE III

Growth Factors and Interleukins

Recombinant growth factors and interleukins were provided as a gift from DNAX (Palo Alto, CA). In some experiments LCM was used as a source of M-CSF. The amount of M-CSF present in the LCM was determined using an M-CSF standard obtained from DNAX (Palo Alto, California). The growth activity of LCM could be blocked by a specific

antibody against M-CSF (Cetus Corp., Emeryville, CA). Porcine platelet TGF-ß1 was obtained from RFD Systems, Inc. (Minneapolis, MN).

To determine the effect of TGF-ß on macrophage proliferation, different concentrations of TGF-ß (0.1 to 10 mg/ml) were added to murine bone marrow derived macrophages ("BMM") cultured in the presence of M-CSF for seven days. Macrophages were washed and incubated at a concentration of 1 x 10^6 cells/ml in a 24-well plate. These cells were grown from bone marrow cultures in the presence of M-CSF in the amounts indicated in Figure 1, which allowed macrophages to proliferate (>99% are Mac1 positive), while the rest of the bone marrow cells died. Each determination was made in triplicate, and the points shown in Figure 1 represent the mean.

In the absence of M-CSF, TGF-ß did not significantly enhance macrophage proliferation. As shown in Figure 1, when TGF-ß was added to these differentiated macrophages in the presence of recombinant M-CSF, thymidine incorporation increased in a dose-dependent manner. Amounts of TGF-ß over 10 µg/ml (up to 100 ng/ml) did not significantly increase thymidine incorporation further. When cells were counted in a parallel experiment, an increase in cell number in cultures treated with TGF-ß plus M-CSF was seen when compared to cultures treated with M-CSF alone. A significant difference was observed using 600 or 1200 U/ml of M-CSF, when the controls were compared with samples treated with 1 or 10 ng/ml of TGF-ß ($p<0.01$). These data suggest that TGF-ß stimulates the M-CSF dependent proliferation of BMM.

The effect of TGF-ß on the proliferation of macrophages at different stages of maturation was also tested. Nonadherent macrophages were obtained after 3 days in culture in the presence of M-CSF. After washing,

nonadherent macrophages or elicited peritoneal macrophages were incubated in the presence of 1200 U/ml of M-CSF, and the amounts of TGF-ß indicated in Figure 2. Each determination was made in triplicate and the results are expressed as the mean and 1 SD. A significant difference was observed between the controls and the samples treated with 1, 10 or 50 ng/ml of TGF-ß ($p<0.05$ to $p<0.01$).

The proliferation of nonadherent BMM (3 days in culture), which represent an early stage of macrophage differentiation, was compared with elicited peritoneal macrophages which represent a committed population of cells. The nonadherent BMM incorporated about five times more thymidine than the elicited peritoneal macrophages (Figure 2). In the presence of M-CSF, TGF-ß had a different effect on each cell population. As shown in Figure 2, TGF-ß stimulated the proliferation of elicited peritoneal macrophages (committed cells), it inhibited proliferation of the nonadherent BMM cells (immature macrophages).

To determine if the enhancing effect of TGF-ß was specific only for M-CSF induced proliferation, the growth factors, GM-CSF and IL-3, as well as several other interleukins were tested. The effect of adding TGF-ß to macrophages treated with either of these factors was tested. BMM were obtained after 7 days in culture in the presence of M-CSF. After washing, cells were incubated in the presence of 10 ng/ml of recombinant GM-CSF or IL-3 and the indicated amounts of TGF-ß. A significant difference was observed between the controls and the samples treated with 1, 10 or 50 ng/ml of TGF-ß ($p<0.01$).

Both GM-CSF (10 ng/ml) and IL-3 (10 ng/ml) induced macrophage proliferation but GM-CSF was more active than IL-3 (Figure 3). In a dose-dependent fashion, TGF-ß enhanced the activity of GM-CSF, but had a moderate

suppressive effect on IL-3 dependent proliferation. Other factors such as IL-1, 4 and 6 but not IL-2, had a small positive effect on BMM proliferation. When TGF-ß was added together with these factors, there was no significant change in proliferation.

EXAMPLE IV

Effect of Anti-TGF-ß Antibodies

The TGF-ß blocking antibody was a gift of Dr. E. Ruoslahti (La Jolla Cancer Res. Fdn., La Jolla, CA) and was prepared by immunizing two rabbits with 0.5 mg each of a synthetic peptide coupled to methylated bovine serum albumin in complete Freund's adjuvant. The antibody is described in Okuda et al., J. Clin. Invest. 86:453 (1990). The peptide was synthesized from the amino acid sequence 78-109 of mature human TGF-ß1. The rabbits were boosted at 4-week intervals and bled via the ear artery. The antibody was affinity purified by standard conventional procedures.

TGF-ß is able to upregulate its own expression in macrophage. The effect of this autocrine production of TGF-ß on macrophage proliferation was examined to determine the effect of suppressing the activity of TGF-ß would have on the proliferation of macrophages.

BMM were treated with recombinant growth factors and with a blocking antibody made against a synthetic peptide derived from TGF-ß as described in Okuda et al., supra. As shown in Figure 4, the immune antiserum, but not the preimmune, inhibited the M-CSF and GM-CSF macrophage dependent proliferation (32% and 28%, respectively; $p<0.01$), but there was no effect on IL-3 dependent proliferation. These data suggest that the proliferation induced by M-CSF and GM-CSF involves the production of TGF-ß, which then contributes to the proliferative response. In addition, to inhibit the

proliferation of macrophages, the data suggest that an an agent that suppresses the activity of TGF-ß can used.

Although the invention has been described with reference to various embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. A method for stimulating the proliferation of differentiated macrophages, comprising contacting the differentiated macrophages with an effective amount of a macrophage-stimulatory TGF-β.

2. The method of claim 1, wherein the differentiated macrophages are contacted with an effective amount of a colony stimulating factor and an effective amount of TGF-β to stimulate the proliferation of the macrophages.

3. The method of claim 2, wherein the colony stimulating factor is M-CSF.

4. The method of claim 2, wherein the colony stimulating factor is GM-CSF.

5. A method for inhibiting the proliferation of differentiated macrophages, comprising contacting the differentiated macrophages with an effective amount of an agent which modulates the activity of a macrophage-stimulatory TGF-β.

6. The method of claim 5, wherein the agent is an antibody having specific reactivity with TGF-β.

7. The method of claim 5, wherein the agent is decorin or a functional equivalent.

8. The method of claim 7, wherein the functional equivalent is biglycan.

9. A method for inhibiting the proliferation of immature macrophages induced to proliferate with a colony stimulating factor, comprising contacting the immature macrophages with an effective amount of a macrophage-inhibitory TGF-β to inhibit the proliferation.

10. The method of claim 9, wherein the colony stimulating factor is M-CSF.

11. The method of claim 9, wherein the colony stimulating factor is GM-CSF.

12. A method for stimulating the proliferation of immature macrophages not induced to proliferate with a colony stimulating factor, comprising contacting the immature macrophages with an effective amount of a macrophage-stimulatory TGF-β to stimulate proliferation of the immature macrophages.

13. A method for inhibiting the proliferation of undifferentiated macrophages, comprising contacting the undifferentiated macrophages with an effective amount of macrophage-inhibitory TGF-β.

14. A method for treating an individual with a condition characterized by the overabundance or lack of adequate differentiated macrophages, comprising modulating the amount or activity of TGF-β activity in macrophage-containing tissues of the individual.

15. The method of claim 14, wherein the condition is characterized by the overabundance of differentiated macrophages and the activity of TGF-β is modulated so that the proliferation of the differentiated macrophages is inhibited.

16. The method of claim 15, where the macrophages are contacted with an effective amount of an agent that suppresses the activity of TGF-β.

17. The method of claim 16, wherein the agent is an antibody having specific reactivity with TGF-β.

18. The method of claim 16, wherein the agent is decorin or a functional equivalent.

19. The method of claim 18, wherein the functional equivalent is biglycan.

20. The method of claim 14, wherein the condition is characterized by a lack of adequate differentiated macrophages and the TGF-β is modulated by administering an effective amount of TGF-β and a colony stimulating factor to the individual to stimulate the differentiation of undifferentiated macrophages in the individual.

21. The method of claim 20, wherein the colony stimulating factor is M-CSF.

22. The method of claim 20, wherein the colony stimulating factor is GM-CSF.

23. Use of a macrophage-stimulatory TGF-ß for the manufacture of a medicament for stimulating the proliferation of differentiated macrophages.

24. Use of a macrophage-stimulatory TGF-ß and a colony stimulating factor for the manufacture of a medicament for stimulating the proliferation of differentiated macrophages.

25. The use of claim 24, wherein the colony stimulating factor is M-CSF.

26. The use of claim 24, wherein the colony stimulating factor is GM-CSF.

27. Use of an antibody having specific reactivity with a TGF-ß for the manufacture of a medicament for inhibiting the proliferation of differentiated macrophages.

28. Use of decorin or its functional equivalent for the manufacture of a medicament for inhibiting the proliferation of differentiated macrophages.

29. The use of claim 28, wherein the functional equivalent is biglycan.

30. Use of a macrophage-inhibitory TGF-ß for the manufacture of a medicament for inhibiting the proliferation of immature macrophages induced to proliferate with a colony stimulating factor.

31. The use of claim 30, wherein the colony-stimulating factor is M-CSF.

32. The use of claim 30, wherein the colony-stimulating factor is GM-CSF.

33. Use of a macrophage-inhibitory TGF-ß for the manufacture of a medicament for inhibiting the proliferation of immature or undifferentiated macrophages.

34. Use of a macrophage-stimulatory TGF-β for the manufacture of a medicament for stimulating the proliferation of immature macrophages not induced to proliferate with a colony stimulating factor.

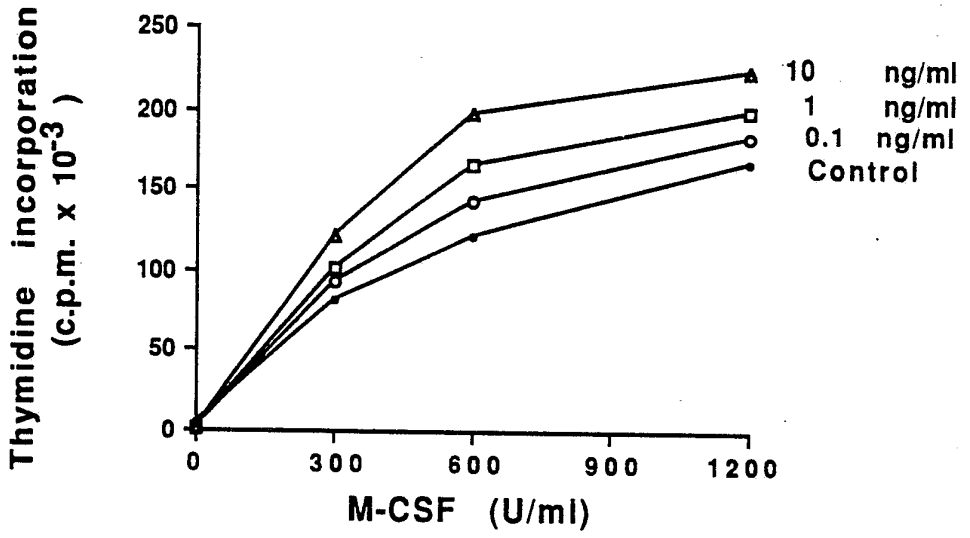
Thymidine incorporation
(c.p.m. x 10^{-3})
250
200
150
100
50
0
0
300
600
900
1200
M-CSF (U/ml)
10 ng/ml
1 ng/ml
0.1 ng/ml
Control

Figure 1

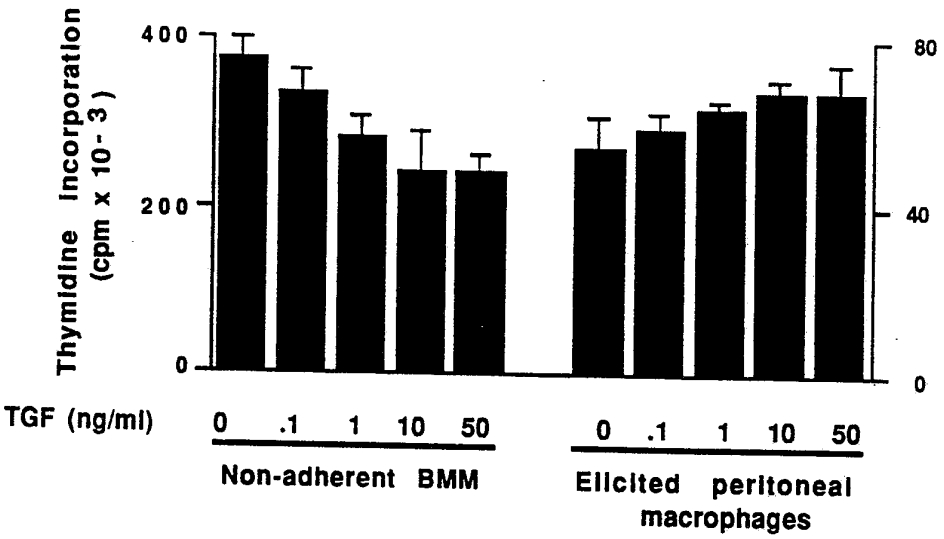
Thymidine Incorporation (cpm x 10-3)
400
200
0
80
40
0
TGF (ng/ml)
0 .1 1 10 50
0 .1 1 10 50
Non-adherent BMM
Elicited peritoneal macrophages

Figure 2

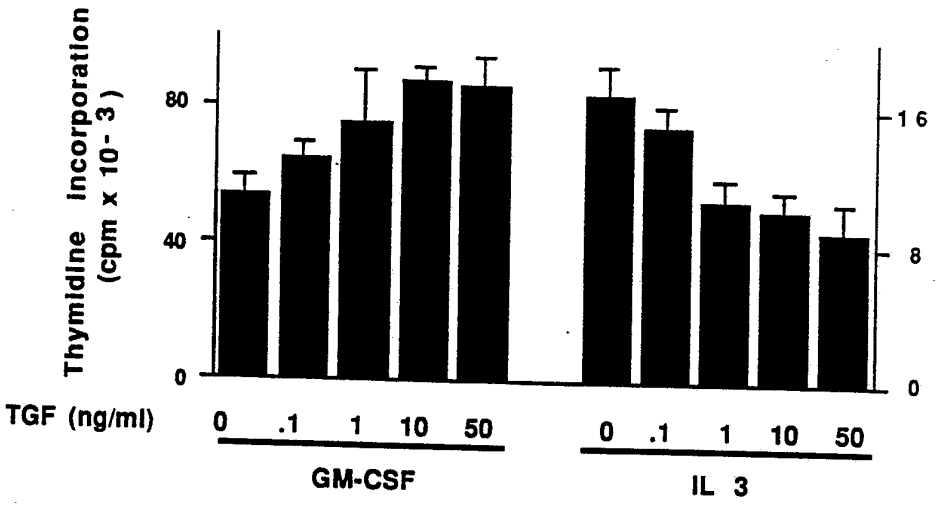
Thymidine Incorporation
(cpm x 10- 3)
80
40
0
16
8
0
TGF (ng/ml)
0 .1 1 10 50
0 .1 1 10 50
GM-CSF
IL 3

Figure 3

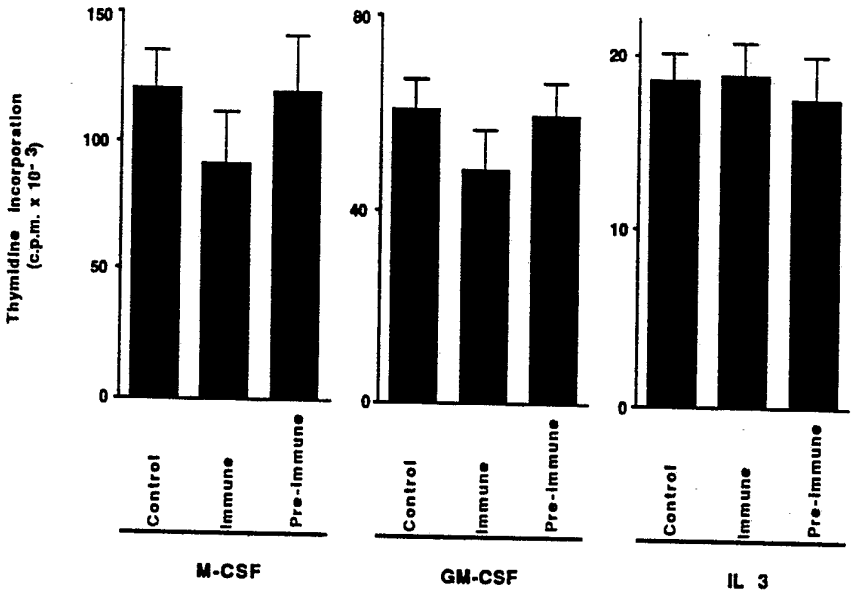
Thymidine incorporation (c.p.m. x 10⁻³)
150
100
50
0
80
40
0
20
10
0
Control
Immune
Pre-immune
M-CSF
GM-CSF
IL 3

Figure 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00998

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 A61K37/02; A61K39/395

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

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	EP,A,0 454 400 (CELTRIX LABORATORIES, INC.) 30 October 1991 see the whole document	12,14, 20-22,34
X	JOURNAL OF IMMUNOLOGY. vol. 140, no. 8, 15 April 1988, BALTIMORE US pages 2645 - 2651 G. STRASSMANN ET AL. 'REGULATION OF COLONY-STIMULATING FACTOR 1-DEPENDENT MACROPHAGE PRECURSOR PROLIFERATION BY TYPE BETA TRANSFORMING GROWTH FACTOR.' see the whole document	9-11,13, 14,30-33
X Y	WO,A,9 115 223 (AMGEN INC. ET AL.) 17 October 1991 see page 9, line 14 - line 18; example 5; table 2	5,6, 14-17,27 7,8,18, 19,28,29

-/--

° 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
21 MAY 1993	21 -06- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	RYCKEBOSCH A.O.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	WO,A,9 110 727 (LA JOLLA CANCER RESEARCH FOUNDATION) 25 July 1991 see claims 1-11	7,8,18, 19,28,29
P,X	JOURNAL OF IMMUNOLOGY. vol. 148, no. 4, 15 February 1992, BALTIMORE US pages 1102 - 1105 A. CELADA ET AL. 'TRANSFORMING GROWTH FACTOR BETA ENHANCES THE M-CSF AND GM-CSF-STIMULATED PROLIFERATION OF MACROPHAGES.'	1-6, 9-17, 20-27, 30-34
P,Y	see the whole document	7,8,18, 19,28,29
P,X	WO,A,9 217 206 (THE VICTORIA UNIVERSITY OF MANCHESTER.) 15 October 1992 see page 26, line 11 - line 20; claims 1-3,7,8	1,5-8, 12, 14-19, 23, 27-29,34

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US93/00998

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-13 (as far as relating to an in vivo method) and 14-22 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. [ ] Claims Nos.:
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. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

4. [ ] 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 claims Nos.:

Remark on Protest

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

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

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9300998
SA 70394

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 21/05/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0454400	30-10-91	US-A-	5147799	15-09-92
WO-A-9115223	17-10-91	AU-A-	7699191	30-10-91
		EP-A-	0477345	01-04-92
		JP-T-	4506363	05-11-92
WO-A-9110727	25-07-91	None		
WO-A-9217206	15-10-92	AU-A-	1436892	02-11-92