AUSTRALIA

Patents Act 1990

PATENT REQUEST : STANDARD PATENT

We being the persons identified below as the Applicants, request the grant of a patent to the persons identified below as the Nominated Persons, for an invention described in the accompanying standard complete specification.

Full application details follow:

[71/70]   Applicant(s)/Nominated Person(s):

Andrew Dale Weinberg AND Arthur Allen Vandenbark

Address:

3266 SW Fairmount Boulevard, Portland, Oregon 97201, United States of America and 4371 SW 48th Place, Portland, Oregon 97221, United States of America, respectively

[54]   Invention Title:

T-cell antigens, and their use in diagnosis and treatment of T-cell mediated conditions II

[72]   Names of actual inventors:

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DIVISIONAL APPLICATION DETAILS

[62]   Original Application No. 15835/95
         Person by whom made: Andrew Dale Weinberg AND Arthur Allen Vandenbark

         Dated this 4th day of November, 1998.

(A member of the firm of DAVIES COLLISON CAVE
for and on behalf of the Applicant/s.)

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NOTICE OF ENTITLEMENT

We,   Andrew Dale Weinberg AND Arthur Allen Vandenbark
of
3266 SW Fairmount Boulevard, Portland, Oregon 97201, United States of America

and 4371 SW 48th Place, Portland, Oregon 97221, United States of America, respectively

the Nominated Persons/Applicants in respect of the accompanying patent request state the following:-

The Nominated Persons are entitled to the grant of the patent because the Nominated Persons are the inventors.

The Nominated Persons are the applicants of the original application with respect to which the present application is a further complete application under S.39 of the Patents Act 1990.

DATED this 4th day of November, 1998.

....
a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the applicant

(12) PATENT ABSTRACT   **(11)** Document No. **AU-A-91342/98**

(19) AUSTRALIAN PATENT OFFICE

(54) Title
T-CELL ANTIGENS, AND THEIR USE IN DIAGNOSIS AND TREATMENT OF T-CELL MEDIATED CONDITIONS II

International Patent Classification(s)
(51)[6] **G01N 033/68**   **G01N 033/577**

(21) Application No. : **91342/98**   (22) Application Date : **04/11/98**

(43) Publication Date : **21/01/99**

(62) Related to Division(s)   : **15835/95**

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(57)

The present invention is also based on the discovery that

a particular protein antigen, termed OX-40 (herein

referred to as the OX-40 antigen), is specifically

expressed on the cell surface of antigen activated T-

cells especially for example activated CD4^{+} T-cells. In

particular, using the EAE disease model in rats, this

antigen was shown to be expressed on the surface of

activated autoantigen-specific CD4^{+} T-cells present at the

site of inflammation (the spinal cord in this disease

model) but absent on CD4^{+} T-cells at non-inflammatory

sites. Furthermore, the highest expression of this

1.   A method of detecting a condition mediated by activated
T-cells, eg CD4⁺ T-cells, in a patient, comprising contacting
a specific binding agent with said T-cells and quantitating
the level of activated T-cells, wherein said specific
binding agent is a specific binding partner able to bind a
polypeptide having an amino acid sequence encoded by the
nucleic acid sequence shown in SEQ ID No 1 or by bases 15-
848 thereof or other sub-sequence thereof which encodes an
antigenic determinant, or a variant or allele or derivative
thereof.

2.   A method according to claim 1 wherein the specific
binding agent is a specific binding partner able to bind a
polypeptide other than a protein consisting of the full
sequence of human gp34 protein as defined in S. Miura et al.
Mol. Cell Biol. 11(3)(1991) pp 1313-1325.

24.   A kit for treating a condition mediated by activated T-
cells, eg activated CD4⁺ T cells, said kit comprising a
container containing a suitable amount of a specific binding
agent antibody according to any of claims 2 to 13 in a
pharmaceutically acceptable excipient.

AUSTRALIA

PATENTS ACT 1990

DIVISIONAL APPLICATION

NAME OF APPLICANT(S):

Andrew Dale Weinberg AND Arthur Allen Vandenbark

ADDRESS FOR SERVICE:

DAVIES COLLISON CAVE
Patent Attorneys
1 Little Collins Street
Melbourne, 3000.

INVENTION TITLE:

T-cell antigens, and their use in diagnosis and treatment of T-cell mediated conditions II

The following statement is a full description of this invention, including the best method of performing it known to us:

T-CELL ANTIGENS, AND THEIR USE IN DIAGNOSIS AND TREATMENT OF T-CELL MEDIATED CONDITIONS-II

TECHNICAL FIELD

5   This invention relates to methods for the specific depletion of activated T-lymphocytes particularly those belonging to the CD4$^+$ subclass. Such activated T-lymphocytes e.g. CD4$^+$ T-lymphocytes, are implicated in a number of conditions in humans including multiple sclerosis and transplant rejection. In particular, this invention provides a treatment in which activated T-lymphocytes e.g. CD4$^+$ T-cells involved in a particular disease or condition are depleted while the non-activated T-lymphocyte e.g. CD4$^+$ T-cells repertoire is unaffected.

BACKGROUND OF INVENTION

The CD4$^+$ T-lymphocyte (herein referred to as the CD4$^+$ T-cell) is the central player in the immune system because of the "help" it provides to other leukocytes in fighting
20   off infection and potential cancerous cells. CD4$^+$ T-cells play essential roles in both humeral and cell-mediated immunity and additionally they act during parasite infection to promote the differentiation of eosinophils and mast cells. If the CD4$^+$ T-cell population is depleted
25   (as is the case in AIDS patients) the host is rendered susceptible to a number of pathogens and tumours that do not ordinarily pose a threat to the host.

While CD4⁺ T-cells thus play an important beneficial role
in disease prevention, the aberrant function of these
cells can produce serious problems. In some individuals,
the aberrant function of CD4⁺ T-cells leads to

5   autoimmunity and other disease states (Swanborg, R.H.,
1984; Cush, J.J., and Lipsky, P.E., 1988; Caspi et al.,
1988). Autoimmune diseases in which CD4⁺ T-cells have
been implicated include multiple sclerosis, rheumatoid
arthritis and autoimmune uveitis (see generally,

10   Steinman, L., 1993). In essence these diseases involve
an aberrant immune response in which the immune system is
subverted from its normal role of attacking invading
pathogens and instead attacks the host body tissues,
leading to illness and even death. The targeted host

15   tissues vary between autoimmune diseases, for example, in
multiple sclerosis the immune system attacks the white
matter of the brain and spinal cord, in rheumatoid
arthritis the immune system attacks the synovial lining
of the joints. Activated CD4⁺ T-cells have also been

20   implicated in other illnesses, including rejection of
transplant tissues and organs and in the development of
CD4⁺ T-cell lymphomas.

Investigations into conditions caused by aberrant CD4⁺ T-

25   cell activity are focused on several animal models, and
in particular on a number of experimentally induced
autoimmune diseases. Research on these experimentally
induced diseases in animals is premised on the idea that

they will provide information useful in the treatment of
the corresponding human diseases. In pursuit of this
goal, it has been shown that CD4$^+$ T-cells are responsible
for several experimentally induced autoimmune diseases in
5   animals, including experimental autoimmune
encephalomyelitis (EAE), collagen induced arthritis
(CIA), and experimental autoimmune uveitis (EAU).

EAE is induced by autoimmunizing animals against myelin
10   basic protein (MBP, a component of the white matter of
the brain and the spinal cord) and produces the same
clinical symptoms observed in multiple sclerosis:
demyelination and paralysis. Proof of the value of the
EAE model as a comparative model for multiple sclerosis
15   has been provided by evidence showing that these
conditions share a causative nexus: Steinman and co-
workers showed that the predominant cell type found in
the brain lesions of multiple sclerosis patients is CD4$^+$
T-cells (Oksenberg, J.R., et al., 1990) and that the T-
20   cell receptor (the molecule responsible for antigen
recognition) associated with the cells in these brain
lesions had the same 3 amino acid binding motif for
antigen recognition as on the CD4$^+$ T-cells responsible for
causing experimental autoimmune encephalomyelitis (EAE)
25   (Oksenberg, J.R., et al., 1993). All the evidence thus
suggests that the EAE model will be useful in testing
therapies for multiple sclerosis.

Research on a number of the experimentally induced

autoimmune diseases, including EAE, CIA and EAU, has

shown that antibodies that bind CD4⁺ T-cells when injected

in vivo can inhibit the development of these diseases as

5   well as inhibit transplantation rejection (Swanborg, R.H.

1983; Cobbold, S.P. et al., 1984; Steinman, L, 1993).

This antibody-mediated effect depletes or inactivates all

CD4⁺ cells in the body (the antibodies that bind to the

CD4⁺ cells presumably block the activity of the cells and

10   also target the CD4⁺ cells for destruction by the immune

system.) This strategy has shown some success with

rheumatoid arthritis and is now being tested for multiple

sclerosis (see generally, Steinman, L., 1993).

15   While it appears that therapeutic approaches that destroy

the CD4⁺ T-lymphocyte population might be effective in

ameliorating these autoimmune diseases, this approach has

one very major drawback. The treatment not only inhibits

the function of those CD4⁺ T-cells that are antigen

20   reactive and thus involved in the autoimmune disease

process, but also the CD4⁺ T-cells that are quiescent and

not involved in the disease. Since CD4⁺ T-cells are

important in the general immune response (protecting the

body against infectious agents), destruction of the

25   entire CD4⁺ T-cell population leaves the patient severely

immunocompromised and hence highly susceptible to

infection. A preferable approach would be to remove only

those CD4⁺ T-cells that are actively involved in the auto-

immune response, leaving the remaining CD4⁺ T-cell
population available for their normal role in the immune
system.

5   This method of treatment has not yet been achieved. It is
therefore an object of the present invention to provide a
method of specifically depleting the population of
activated CD4⁺ T-cells in a patient without affecting the
quiescent CD4⁺ T-cell population.

10

SUMMARY OF THE INVENTION
In one aspect the present invention provides a method by
which autoantigen specific T-cells, especially for
example activated CD4⁺ T-cells, can be specifically
15   eliminated *in vivo*, while leaving the quiescent
population of T-cells especially for example unactivated
CD4⁺ T-cells intact. This invention therefore provides a
·treatment useful for T-cell mediated especially for
example activated CD4⁺ T-cell mediated autoimmune diseases
20   such as multiple sclerosis, rheumatoid arthritis,
sarcoidosis and autoimmune uveitis, graft versus host
disease (GVHD) and/or inflammatory bowel disease. This
invention also provides a method for eliminating other
undesired immune responses caused by activated T-cells
25   especially for example activated CD4⁺ T-cells such as
rejection of transplanted tissue and organs in transplant
recipients. Furthermore, the present invention provides
a method of specifically eliminating activated CD4⁺ T-cell

lymphomas from the body. The present invention also provides a method for early diagnosis of conditions mediated by activated T-cells especially for example activated CD4⁺ T-cells by detecting the presence of

5   autoreactive T-cells at the site of autoimmune lesions and potentially harmful T-cell lymphomas. This early diagnosis provides an indication that the methods of treatment provided by the present invention may be effective and can facilitate earlier treatment of the

10 condition than might otherwise be possible.

The present invention is also based on the discovery that a particular protein antigen, termed OX-40 (herein referred to as the OX-40 antigen), is specifically

15 expressed on the cell surface of antigen activated T-cells especially for example activated CD4⁺ T-cells. In particular, using the EAE disease model in rats, this antigen was shown to be expressed on the surface of activated autoantigen-specific CD4⁺ T-cells present at the

20 site of inflammation (the spinal cord in this disease model) but absent on CD4⁺ T-cells at non-inflammatory sites. Furthermore, the highest expression of this antigen on these CD4⁺ T-cells was found to occur on the day prior to initiation of clinical signs of

25 autoimmunity; the expression of this antigen decreased as the disease progressed. The specificity of expression of the OX-40 antigen and the transient nature of this expression, shown for the first time in the present

invention, motivated the testing of this antigen as a possible target for antibody mediated depletion of activated T-cells in animals such as humans with T-cell mediated conditions.

5

The applicants have cloned and sequenced the cDNA encoding the OX-40 antigen. Thus the invention provides a nucleic acid having the sequence shown in Seq ID No 1 or a sub-sequence of SEQ ID No 1 which encodes an

10 antigenic polypeptide; or a variant or allele thereof; or a complementary strand to any of these. A particular sub-sequence of SEQ ID No 1 comprises nucleotide bases 15 to 848 shown in Seq ID No 1 or its complementary strand.
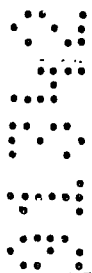
15 Further according to the invention there is provided a polypeptide comprising an amino acid sequence encoded by a nucleic acid as described above or a derivative thereof. Suitably the polypeptide comprises an amino acid sequence encoded by a sub-sequence of the sequence

20 shown in SEQ ID No 1 and which includes an antigenic determinant.
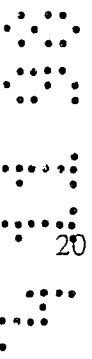
Among the variants of nucleic acid sequences and polypeptides contemplated by the invention are (for

25 example) DNA sequence variants importing no change in encoded amino acid sequence. Then there are (for further example) sequence variants importing "conservative" amino acid changes eg changes from one acidic amino acid to

another, one aromatic amino acid to another, one basic

amino acid to another, one aliphatic hydrophobic

sidechain to another, as is well known in the art. Then

there are for example variants corresponding to allelic

5   variants in the encoded polypeptide, and other variants

that result in polypeptides of antigenic cross-reactivity

and/or similar binding specificity.

The antigenicity of the polypeptides and variants

10   mentioned in the specification includes for example

antigenic determinants shared or cross-reactive with the

OX-40 antigen as encoded by Seq ID No. 1, eg antigenic

polypeptides with determinants that are shared with

determinants of OX-40 that are accessible to specific

15   binding agents when the OX-40 is present on a cell

surface.

Simply finding a target antigen on a particular cell type

does not provide a basis for a therapeutic approach which

20   requires depleting the particular cell type. Thus, many

antigens are shed from the cell surface and are not

suitable as targets for therapy. A further aspect of

this invention is the discovery that a specific binding

agent such as an antibody raised against the OX-40

25   protein and conjugated to a cytotoxin can inhibit the *in

vitro* proliferation of antigen activated CD4$^+$ T-cells.

This discovery implies that the OX-40 antigen is rapidly

internalized by CD4$^+$ T-cells. Additional research based

on this discovery led to an important focus of the
present invention; a demonstration that a population of
antigen activated CD4$^+$ T-cells can be depleted *in vivo* by
conjugating a specific binding agent such as an antibody
5   raised against the OX-40 antigen with a cytotoxin to
produce an immunotoxin, and administering this
immunotoxin to a host. In this manner, the antibody
binds to the OX-40 antigen on the surface of the
activated CD4$^+$ T-cell. Internalization of the immunotoxin
10   results in the cytotoxin being taken into the cell, which
produces cell death. Hence, administration of this
immunotoxin to a host suffering from activated T-cell (eg
CD4$^+$ T-cell) mediated inflammation depletes (or otherwise
inactivates) the activated T-cells especially for example
15   activated CD4$^+$ T-cells at the site of inflammation or
other sites, leading to amelioration of subsequent
inflammation and/or other clinical signs of disease.

A further aspect of the present invention is therefore a
20   method of treating a patient suffering from a condition
mediated by activated T-cells e.g. CD4$^+$ T-cells, which
comprises administering to the patient an effective
amount of a specific binding agent which can specifically
bind to a polypeptide as described above, eg an antibody
25   conjugated with a cytotoxic agent wherein the antibody
recognises and binds to the OX-40 antigen present on the
surface of the T-cells, especially for example activated
CD4$^+$ T-cells.

Particular methods of the invention include a method for

reducing a population of T-cells, e.g. CD4⁺ T-cells that

express an OX-40 antigen in a human host and a method of

inhibiting relapsing autoimmune inflammation in a patient

5   suffering from multiple sclerosis, which methods comprise

administering an effective amount of a specific binding

agent of the invention.

In particular the specific binding agent used in these

10   methods is an antibody conjugated with a cytotoxic agent

(an immunotoxin) wherein the antibody recognizes and

binds to the OX-40 antigen present on the surface of the

T-cells especially for example activated CD4⁺ T-cells.

Alternatively the method may employ a specific binding

15   agent which comprises a Fab, F(ab')2, or Fv fragment of a

monoclonal antibody capable of recognising Ox-40 antigen

when expressed on the surface of T-cells. Other specific

binding agents useful in this method are immunoglobulins

capable of cytotoxic effect on cells bearing Ox-40 on

20   their surface or any specific binding agents which can

fix, complement or mediate antibody-dependant cellular

cytotoxicity such as a specific binding agent which has

or is linked to structure characteristic of the Fc region

of an immunoglobulin of murine type IgG2a or human type

25   IgG1 or IgG3.

The methods of the invention are applicable to any

condition mediated by activated T-cells especially for

example activated CD4⁺ T-cells, including, multiple
sclerosis, sarcoidosis, rheumatoid arthritis, autoimmune
uveitis, T-cell lymphomas and rejection of a transplanted
organ or tissue. Additional conditions to which this

5   method is applicable include graft-versus-host disease or
reaction and inflammatory bowel disease.

T-cells can be activated by for example antigens,
superantigens, mitogens, or monoclonal antibodies.

10

The methods of treatment set forth in the preceding
paragraph will preferably be performed using specific
binding agents such as monoclonal antibodies, or
fragments thereof, which can be raised using the

15   polypeptides of the invention. In a more preferred
embodiment, the monoclonal antibody will be a humanized
monoclonal antibody. In alternative embodiments, the
method will utilize a cytotoxic conjugate, eg comprising
a fragment such as a Fab, F(ab')$_2$ or Fv fragment of a

20   monoclonal antibody conjugated with a cytotoxic agent
wherein the fragment of the monoclonal antibody
recognizes the OX-40 antigen.

This invention also encompasses specific binding agents

25   such as monoclonal antibodies having a specificity of
binding in cells to substantially only antigen activated
T-cells especially for example activated CD4⁺ T-cells. In
a preferred embodiment, the specific binding agents can

specifically bind to a polypeptide of the invention.
Particular specific binding agents specifically bind to
human Ox-40 of amino acid sequence encoded by the coding
region of nucleic acid sequence Seq ID No 1, when said

5   Ox-40 is present on the surface of activated cells.

These specific binding agents suitably comprise an
antibody binding domain and are preferably monoclonal
antibodies or binding fragments thereof. As mentioned

10   above, the antibodies will preferably be at least
partially humanised, and so most preferably comprise a
humanised monoclonal antibody.

"Specific binding" refers for example to specific non-
covalent molecular binding such as that between an
antibody and a corresponding antigen or hapten (its

15   "binding partner") and also between other specialised
binding molecules and their binding partners.

"At least partly humanised", relating to antibodies and

20   their binding domains, means that for example embodiments
are contemplated in which only the constant region (CH
and CL) may correspond to human polypeptide:
alternatively, both the constant and variable regions may

25   be "humanised".

Another aspect of this invention provides a specific
binding agent such as a monoclonal antibody as described

above which further comprises a molecularly linked, eg covalently conjugated, cytotoxin. Antibody-cytotoxin conjugates (also known as immunotoxins) are suitable for use in the methods of treatment described above.

5   Examples of such specific binding agents include Fab, F(ab')$_2$ or Fv fragments of a monoclonal antibody conjugated with a cytotoxic agent.

For therapeutic use, the specific binding agents of the
10   invention are suitably administered in the form of a pharmaceutical composition which include a pharmaceutically acceptable carrier. The carrier may be solid or preferably liquid carriers such as water or saline, which are conventional in the art.
15

A further aspect of the invention comprises a method of detecting a condition mediated by activated T-cells, e.g CD4$^+$ T-cells, in a patient comprising contacting a specific binding agent as described above with said T-
20   cells and quantifying the level of activated T-cells. Suitably the method is carried out on a biopsy sample from the patient, such as a skin or intestinal biopsy sample or a blood sample from a patient suspected of having a graft-versus-host disease, or an intestinal
25   biopsy sample from a patient suspected of having an inflammatory bowel disorder, or a sample of cerebrospinal fluid.

A further aspect of the present invention is a method of detecting an inflammatory condition mediated by activated T-cells especially for example activated CD4$^+$ T-cells in a patient by obtaining a suitable biopsy sample from the patient and then quantifying the percentage of activated T-cells especially for example activated CD4$^+$ T-cells in the biopsy sample using a specific binding agent such as an antibody that specifically binds to the OX-40 antigen. Other aspects of the present invention include test kits for detecting conditions mediated by activated T-cells especially for example activated CD4$^+$ T-cells, and treatment kits comprising antibody in pharmaceutically administrable forms and amounts with suitable excipients and containers.

In particular the invention provides a kit for detecting a condition mediated by activated T-cells e.g. CD4$^+$ T-cells in a patient comprising a specific binding agent as described above which is labelled. Preferably the invention provides a kit for carrying out a specific binding assay for detection or quantitation of an analyte that comprises a polypeptide or a specific binding agent as described above, wherein said kit comprises a first reagent comprising a specific binding agent that can recognise the analyte, a second reagent comprising a substance that can bind specifically either to the analyte or to the first reagent, and a label for the second reagent.

In such a kit the first reagent can comprise an antibody
specific for the analyte, and the second reagent
comprises a labelled antiglobulin specific for the first
reagent.

5

Alternatively the first reagent can comprise an
immobilised specific binding reagent for the analyte,
then the second reagent is a specific binding agent that
can bind to the analyte when the analyte is also bound to
10   the first reagent.

In a further embodiment wherein the second reagent
comprises a substance able to compete with analyte for
binding to the first reagent.

15

For use in this method, the specific binding agent may
further comprises a label such as a radioactive label or
a fluorescent label and-these specific binding agents
form a further aspect of the invention.

20

The specific binding agents of the invention may be
cloned and sequenced in the usual way. Thus the
invention further provides a nucleic acid sequence
encoding the amino acid sequence of a specific binding
25   agent as described above.

Recombinant technology may be used to prepare both the
polypeptides and the specific binding agents of the

invention. Thus appropriate expression vectors, transformed host organisms and methods of preparation which include culturing a host organism form further aspects of the invention.

5

The OX40 binding agent, for instance an anti-OX40 monoclonal antibody, can be administered to those patients suffering from a disease mediated by activated T cells e.g. graft-versus-host disease. The amount administered will depend on the amount required to produce an improvement, either partial or total, in the patient's symptoms. This will depend not only on the severity of the condition and route of administration but also on the administration of other therapeutic agents (eg glucocorticoids, cyclosporine A, prednisolone). The OX40 binding agent may be injected either sytemically (eg intravenous) or locally (eg intramuscular). As discussed elsewhere the OX40 binding agent may be coupled to a toxic substance for maximum therapeutic effect.

OX-40 and Graft Versus Host Disease (GVHD)

In the case of GvHD, immunocompetent T cells derived from the donor tissue or cells attack recipient tissue including skin, gut and liver, which are severely compromised in their ability to carry out their normal function. Such attacks, if not controlled, can lead to death of the patient. The therapeutic agents of the present invention could be used to block the activation

of or to eliminate the donor T cells thereby preventing or halting the disease process.

OX-40 and G~~~: diagnostics

5 The present invention can be used in diagnostic tests and procedures in vitro. For instance the OX40 binding agent can be used to determine the presence of OX40+ T cells in a biopsy sample from a patient. The biopsy sample may be a tissue sample or a sample of blood. Mononuclear cells

10 are isolated from the blood or tissue according to standard techniques (see Practical Immunology. L. Hudson and F.C. Hay, eds. Blackwell Scientific Publications, Oxford) and stained with an anti-OX40 antibody or OX40 binding agent fusion protein. The presence of the OX40

15 binding agent is then detected with an anti-globulin reagent coupled with a fluorochrome such as fluoroscein isothiocyanate or phycoerythrin a..d the number of positive cells analysed on a flow cytometer or by fluorescence microscopy (see eg Practical Flow Cytometry.

20 Shapiro, H.M., ed. Alan R Liss, New York; Practical Immunology. L. Hudson and F.C. Hay, eds. Blackwell Scientific Publications, Oxford). Alternatively the tissue sample is processed for immunohistochemical staining by standard techniques (see eg

25 Immunocytochemistry: Practical Applications in Pathology and Biology. J. Polak and S. van Noorden, eds. John Wright and Sons, Bristol). The OX40+ population of cells could be further characterised by two- or three-colour

flow cytometry or immunohistochemistry (see eg Practical Flow Cytometry. Shapiro, H. M., ed. Alan R. Liss, New York; Immunocytochemistry: Practical Applications in Pathology and Biology. J. Polak and S. van Noorden, eds. John Wright and Sons, Bristol). Detection of OX40+ cells can aid in the diagnosis and management of diseases caused by activated T cells e.g. inflammatory bowel disease and GvHD and may be used to follow the course of the disease: an increase in the proportion of OX40+ cells would suggest a worsening of the disease and may indicate the need to increase the dose of therapeutic agent being administered, while a decrease in the proportion of OX40+ cells would suggest an improvement and thus indicate a diminution in the amount of therapeutic agent being administered.

Determination of the levels of OX40+ cells in the blood of patients at risk of GvHD (eg following allogenic bone-marrow transplantation) may allow one to predict the imminent onset of GvHD. Early administration of immunosuppressive agents to control GvHD will improve the likelihood of successful treatment.

The present invention can be used in diagnostic tests and procedures in vivo. For instance, the administration of an OX40 binding agent coupled to a radioisotope can be used for the purposes of immunoscintigraphy.

These and other aspects of the present invention will become more readily apparent from the following figures and description of the invention.

5   DESCRIPTION OF THE DRAWINGS

Fig. 1 shows dot plots from a fluorescence activated cell sorting (FACS) apparatus showing tissue specific dual expression of RT7.2 and the MRC OX-40 antigen. Lymphocytes were isolated from the various tissue

10   compartments designated in the figures during the onset of EAE. The cells were stained with the OX-40 antibody conjugated to fluorescein isothiocyanate (FITC) displayed on the x-axis and counterstained with a R-phycoerythrin (PE) conjugated to the RT7.2 antibody

15   displayed on the y-axis. An isotype matched control antibody was used to draw the quadrants for both the FITC and PE conjugated Abs. The OX-40 antibody was 50% positive on the donor T-cells isolated from the spinal cord, and 8, 2, and 1.8% positive for the donor cells

20   isolated from the CSF, spleen, and blood respectively.
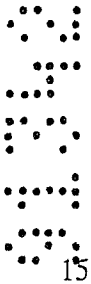
Fig. 2 shows dot plots from a FACS apparatus showing a time course of OX-40 expression on donor T-cells isolated from the spinal cords of rats with EAE.

25   Lymphocytes were isolated from the spinal cords of rats during the time course of disease. The cells were stained with the OX-40 antibody (FITC) and counter-stained with a PE conjugated RT7.2 antibody. An isotype

matched control antibody was used to draw the quadrants
for both the FITC and PE conjugated Abs. The day of the
EAE time course for each dot plot is indicated on the top
of the graphs and the disease score (the severity of the

5   clinical signs and disease score is provided in the
methods section under "Adoptive Transfer of EAE") is
shown in parenthesis next to the day of disease. The
percentage of RT7.2$^+$ cells were 77, 75, 81, 37, and 52%
respectively for Days 0, 1, 2, 3, and 5 after disease

10   onset. The OX-40 antibody was 54% positive for the donor
T-cells on Day 0 (day before onset) and 41, 30, 18, and
12% positive on days 1, 2, 3, and 5 respectively. On Day
5 the EAE score of "+" means the animal had minimal
clinical signs of paralysis (less than 1) but was not

15   completely well.

Figure 3 shows TCR Vβ expression in OX-40 positive and
negative sorted populations isolated from the spinal
cords of Lewis rats with EAE. Lewis rats were actively

20   immunised with myelin basic protein in CFA. Spinal cord
lymphocytes were isolated at the onset of disease. The
cells were immediately lysed after the OX-40 separation,
the RNA was isolated, and analysed for Vβ T cell receptor
gene usage. In the bottom two panels the cells were

25   sorted with the FACStar (Becton-Dickenson, San Jose, CA),
while the top panel compares the unsorted population.
The unsorted cell population was stained with the Vβ8.2-
FITC antibody and showed a similar positive percentage as

observed with the PCR data in panel A.

Fig 4 shows antigen response to OX-40 positive cells isolated from the spinal cord and lymph node. Lewis rats were actively immunised with MBP in CFA and the spinal cord lymphocytes and lymph node cells were isolated at the onset of EAE. The cells were sorted into OX-40$^+$ and OX-40$^-$ fractions, cultured in IL-2 for 5 days, and were stimulated with the corresponding antigen. The unsorted spinal cord population was cultured in IL-2 for 5 days and assayed directly. The cells were incubated with irradiated thymocytes (APC) and antigen and 3[H]-Thymidine was added 48 hr later. Triplicate wells were harvested onto glass fiber filters 18 hr after the label was added. All of the cell types (except the unsorted population) were stimulated with Con A as a control and showed approximately 100,000 CPMs for each group (data not shown).

Fig. 5 is a graph showing dose dependent inhibition of antigen specific CD4$^+$ T-cell proliferation by the OX-40-dgA conjugate. Varying concentrations of the OX-40 immunotoxin was added to a constant amount of F1 T-cells, antigen presenting cells (APC), and MBP (myelin basic protein antigen). The open bars (to the right of the graph) show the proliferation (represented by ['H]-thymidine incorporation) of these T-cells with and without antigen with no immunotoxin added (Pos and Neg

respectively). The assay was carried out in a 200 μl
volume.

Fig. 6A and Fig. 6B are two graphs showing dose dependent
inhibition of antigen specific CD4⁺ T-cell proliferation
by the OX-40-exotoxin conjugate. Varying concentrations
of the OX-40-exotoxin was added to a constant amount of
F1 T-cells (Fig. 4A) or Lewis T-cells (Fig. 4B), APC, and
MBP. The open bars (to the right of the graphs) show the
proliferation (represented by [³H]-thymidine
incorporation) of these T-cells with and without antigen
(Pos and Neg respectively). The assay was carried out in
a 200 μl volume.

Fig. 7 is a graph showing dose dependent inhibition of
PPD antigen specific CD4⁺ T-cell proliferation by the
OX-40-dgA conjugate. Varying concentrations of the OX-40
immunotoxin was added to a constant amount of F1 T-cells,
antigen presenting cells (APC), and PPD (antigen). The
open bars (to the right of the graph) show the
proliferation (represented by [³H]-thymidine
incorporation) of these T-cells with and without antigen
(Pos and Neg respectively). The assay was carried out in
a 200 μl volume.

Fig. 8A and Fig. 8B are two dot plots from a FACS
apparatus showing characterization of lymphocytes
isolated from the spinal cord of rats that had been

treated with a 400 μg dose of OX-40-dgA (Fig. 6A) or

untreated rats (Fig. 6B). F1 MBP specific CD4⁺ T-cells

were transferred into irradiated Lewis recipients and

OX-40-dgA was given on the same day of transfer. The

5 transfer population was detected by the RT7.2 antibody

conjugated to PE (represented on the y-axis) and

counterstained with the control antibody anti-rat

IgM-FITC.

10 Figure 9 shows OX-40 immunotoxin treatment administered

on the first day of disease onset. F1 MBP specific T

cells were transferred into 8 irradiated Lewis rats. On

the first day of disease onset 4 of the animals were

treated with 400 μg of OX-40 immunotoxin and were

15 compared to the untreated "Control" animals. After

treatment, both groups were scored daily until the

clinical signs of EAE subsided. Each point on the graph

represents the mean clinical score of 4 animals for the

treated and control groups. The clinical scores were

20 treated on the same scale as described in previous

figures.

Figure 10 shows effect on donor positive (MBP specific) T

cells isolated from the spinal cord of animals treated

25 with OX-40 immunotoxin. Irradiated Lewis rats were

injected with F1 (Lewis x Buffalo) MBP specific T cells

(1 x 10⁷ cells/animal). In panels A, C, and E the animals

were used as "controls" and received encephalitogenic

cells alone. In panels B, D, and F the animals received
encephalitogenic cells plus a single dose of OX-40
immunotoxin i.p. (400 µg/animal). Panel B shows the
effect of the treatment given on the same day of cell

5   transfer, while panels D and F show the effect of
treatment given the day before disease onset (4 days
after cell transfer). All the animals were sacrificed on
the first day of disease onset which was 24 hr after
treatment in panels D and F. The spinal cord lymphocytes

10   were then stained with RT7.2-PE and the percent positive
cells are shown in the top right corner. The total
number of RT7.2$^+$ spinal cord lymphocytes are also shown
and was calculated by multiplying the percentage of RT7.2$^+$
cells by the total number of lymphocytes isolated from
the spinal cord prep. The EAE clinical score is

15   represented in the top left corner and were the animals
were rated according to the following scale: 0, no signs;
1, limp tail; 2, hind leg weakness, ataxia; 3, hind
quarter paralysis; 4, front and hind limb paralysis,
moribund condition. In this scale a half step (0.5)

20   means the animals were in between the severity of the
numbered scale.

Fig. 11 shows the nucleotide sequence of the coding

25   region of the human OX-40 cDNA and the theoretical amino
acid sequence of the human OX-40 antigen. These
sequences are encompassed within SEQ I.D. No. 1 set forth
in the accompanying sequence listing.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides, for the first time, a method of eliminating undesired immune responses caused by antigen activated T-cells especially for example activated CD4^{+} T-cells. Without wishing to be bound by theory, the inventors made the invention after making findings suggestive that a cell surface antigen, herein termed the Ox-40 antigen, is upregulated solely or preferentially on activated T-cells, especially for example activated CD4^{+} T-cells found at the site of inflammation, and that this cell surface antigen appears to be internalized rapidly. Based on this discovery, a therapeutic method has been developed which utilizes antibodies which bind to the OX-40 protein (herein termed OX-40 antibodies) linked to cytotoxins, to destroy cells expressing the OX-40 antigen. This type of therapy will be extremely useful because it is targeted only to activated T-cells especially for example activated CD4^{+} T-cells and leaves the rest of the T-cell repertoire intact.

Activated T-cells especially for example activated CD4^{+} T-cells have been implicated in a number of antigen activated autoimmune diseases, including multiple sclerosis, sarcoidosis, rheumatoid arthritis and autoimmune uveitis, as well as in transplantation rejections. (Swanborg, R.H., 1984; Cush, J.J., and Lipsky, P.E., 1988; Caspi et al, 1988; Cobbold, S.P. et

al., 1984.) CD4⁺ T-cell lymphomas have also been shown to have an activated phenotype (Gootenberg, J.E. et al., 1981). The present invention provides both methods of diagnosis and methods of treatment for these and other conditions mediated by activated T-cells especially for example activated CD4⁺ T-cells. More particularly, and following the description of relevant materials and methods used in this invention, experimental data obtained during the development of the present invention is presented. These data demonstrate that the OX-40 protein is exclusively expressed at the site of autoimmune inflammation in rats with EAE on the surface of myelin basic protein (MBP) activated CD4⁺ T-cells. It is further shown that the proliferation of MBP activated CD4⁺ T-cells can be inhibited *in vitro* using an OX-40 antibody conjugated with a Ricin dgA cytotoxin. This inhibitory activity is shown not to be limited to MBP activated cells, but also to be effective in inhibiting the proliferation of CD4⁺ T-cells activated by other antigens, including an antigen derived from *Mycobacterium tuberculosis*. The OX-40 antibody-cytotoxin conjugate is shown to be effective *in vivo*; use of the conjugate is shown to inhibit the clinical development of EAE. Following this, the cloning of the human OX-40 homolog is presented along with the production of monoclonal antibodies to the human OX-40 protein.

Various examples are presented showing the application of

the present invention. Specifically, Example 1 describes preferred methods of producing the human OX-40 cDNA enabled by the present invention. Example 2 describes methods of producing purified human OX-40 protein, and

5 Example 3 describes the production of monoclonal and polyclonal antibodies that recognize the human protein. Example 4 describes the production of immunotoxins, based on these monoclonal antibodies, that are suitable for therapeutic use in humans, and other antibody conjugates

10 suitable for diagnostic use. Example 5 describes the use of human OX-40 monoclonal antibodies in diagnosing activated CD4$^+$ T-cell mediated conditions and Example 6 describes the use of the immunotoxins in therapeutic applications. Example 7 describes kits for the diagnosis

15 and treatment of activated CD4$^+$ T-cell mediated conditions.

MATERIALS AND METHODS

<u>Animals</u> Lewis and Buffalo rats were obtained from Harlan

20 Sprague-Dawley, Inc., Indianapolis, IN. Twelve week old Lewis females were bred with 12 week old Buffalo male rats to generate the F1 Lewis x Buffalo hybrid animals. These F1 progeny were used at 8 to 12 weeks of age for MBP immunization. The rats were housed under germ-free

25 conditions at the VA Medical Center Animal Care Facility, Portland, OR according to institutional guidelines.

<u>Selection of MBP Specific F1 and Lewis CD4$^+$ Lymphocyte</u>

<u>Lines</u> T lymphocyte lines were selected on day 12 after

immunization with myelin basic protein (MBP). Details of

this procedure were described earlier (Vandenbark, A.A.,

et al., 1985). Briefly, a lymph node cell suspension was

incubated with MBP (30 μg/ml) in RPMI 1640 with 1%

autologous rat serum. After 3 days at 37°C in a 7% CO_2

atmosphere the cells were cultured in RPMI with 10% horse

serum and IL-2. The T-cell lines were maintained in this

medium until the rate of division slowed. At this point

(7-14 days after MBP stimulation) the cells were

restimulated with 10 μg/ml of MBP presented by irradiated

Lewis thymocytes, and subsequently expanded further in

IL-2 containing medium.

<u>Adoptive Transfer of EAE</u> Activation of the F1 or Lewis

T-cell lines for passive transfer of EAE was carried out

in 10 cm² culture dishes using 5 x 10⁶ T-cells, 100 x 10⁶

irradiated APC, and 10 μg/ml MBP in 10 ml of medium.

After three days of activation the blasts were counted

and 5-10 x 10⁶ T-cell blasts were injected with the

associated APC population i.p. into irradiated (600 rads

for the F1 into Lewis transfers) or non-irradiated naive

Lewis rats. The naive allogeneic recipients were

irradiated the day before adoptive transfer. The

recipient rats were inspected daily, and the clinical

signs of disease were recorded and scored as follows EAE:

0 = no signs;

1 = flaccid tail; 2 = ataxia; 3 = hindquarter paralysis;

4 = quadriplegic/moribund.

Cell Collection   Cerebrospinal fluid (CSF) was collected
by performing cisterna magna puncture using a 27 ga x
3/8" needle with 8" tubing (Abbott Hospitals, Inc.,
Chicago, IL).   The CSF was diluted in RPMI 1:4 and viable
cell numbers were counted.   On average, 100 µl/rat were
collected.   Samples were excluded if the RBC/WBC ratio
exceeded 2:1.   The blood was obtained by heart puncture
and the lymphocytes were separated on-Ficoll-Hypaque as
described by Kruisbeek, A.M., 1992.   The spleen cells
were pushed through a wire mesh screen and the RBCs were
lysed by the NH₄Cl method (Kruisbeek, A.M. 1992).

Spinal Cord Lymphocyte Isolation   Spinal cord mononuclear
cells were isolated following a modified version of a
published protocol (Bourdette, D.N. et al., 1991).
Briefly, spinal cords were isolated by insufflation,
washed 3x in RPMI in order to remove any contaminating
blood cells, homogenized, and then passed through a wire
mesh screen.   The cells were then washed and resuspended
in isotonic Percoll (80%).   For each individual spinal
cord a 10 ml step gradient was poured into a 15 ml
conical tube.   Each step gradient had 100% (2 ml), 80% (4
ml), and 40% (4 ml) isotonic Percoll and the cells were
layered as part of the 80% fraction.   The interface
between the 80/40% Percoll steps was harvested and the
cells were directly spun down and washed.   The

lymphocytes obtained at this interface contained both the resting and blasting populations as assessed by forward scatter. A typical yield of lymphocytes obtained from the spinal cord of animals with EAE was usually 0.5-1.5 x

5   10^6 cells. Lymphocyte recovery was fairly consistent throughout the disease time course, and decreased during the last day of the recovery phase of EAE to 1/2 or 1/3 of the maximal cell number.

10   Fluorescence activated cell sorting (FACS) analysis For the dual fluorescence analysis shown in Fig. 1 and 2, the antibodies used were the RT7.2-PE Ab (Pharmingen, La Jolla, CA) and the MRC OX-40-FITC Ab (Pharmingen, La Jolla, CA). All the analysis was performed on a FACScan

15   with the FACScan Research Software Version A (Becton Dickenson, San Jose, CA) operated according to the manufacturer's instructions.

Antigen Specific Proliferation Assays Proliferation

20   assays were performed in 96-well plates as described previously (Vandenbark, A.A. et al., 1985). Briefly, 2 x 10^4 CD4$^+$ T-cells and 1 x 10^6 irradiated thymocytes/well were incubated in RPMI and 1% rat serum along with antigen and varying concentrations of the OX-40

25   immunotoxin or the toxin alone in a 200 µl volume. The cultures were incubated for 72 hr, the last 18 hr in the presence of 0.5 Bq [^{3}H]-thymidine. The cells were harvested onto glass fiber filters and [^{3}H]-thymidine

uptake was assessed by liquid scintillation. Mean cpm were calculated from triplicate wells. The SD from replicate wells varied <10% from the mean values.

5   <u>OX-40 Immunotoxin Inhibition of EAE</u> Naive Lewis rats were injected with an encephalitogenic dose of MBP specific CD4⁺ T-cells injected in one flank and injected with the immunotoxin at the same time in the opposite flank. Originally a dose curve for the immunotoxin was

10   set up and the optimal dose was found to be between 300-500 µg/8 week old rat. As controls the same molar amount of the toxin alone (dgA) was given to animals in parallel.

15   EXPERIMENTAL RESULTS

One of the keys to understanding the mechanism(s) by which autoantigen specific T-cells destroy self-tissue is to study the differences associated with an autoreactive T-cell at the site of inflammation versus a

20   non-inflammatory site. To this end an experimental model system was set up in EAE to detect the cells that cause the disease *in vivo*.

EAE can be induced by the adoptive transfer of *in vitro*-

25   activated MBP specific CD4⁺ T-cells into naive recipient rats. Four days after the transfer the animals start showing the paralytic signs of EAE. To allow detection of the donor population within the host, MBP specific F1

(Lewis x Buffalo) CD4· T-cells were transferred into naive
irradiated Lewis recipients. Lewis and Buffalo rats
express allelic variants of an epitope of the CD45 cell
surface molecule. These allelic variants are termed

5   RT7.1 (Lewis) and RT 7.2 (Buffalo). The RT7.2 allelic
marker can therefore be used to detect the F1 T-cells in
Lewis hosts because these cells express both forms of the
allele while Lewis rats only express the RT7.1 form of
the allele. The CD45 molecule is expressed only on

10   leukocytes and constitutes approximately 10% of the total
surface protein.

Fig. 1 shows that on the first day of disease onset 50%
of the MBP specific transferred population (RT7.2^p) was

15   positive for the activation marker OX-40 at the site of
inflammation (spinal cord), but the transferred
population was negative for this cell surface antigen at
the non-inflammatory sites (blood and spleen). This
suggested that the OX-40 antigen was expressed on the

20   cell surface of autoreactive CD4· T-cells upon antigen
recognition *in vivo*, because the MBP antigen is present
on the T-cells in the spinal cord but not in the blood or
spleen. The highest expression of the OX-40 antigen on
the donor population isolated from the spinal cord was on

25   the day before clinical signs of EAE and as the disease
progressed this cell surface molecule went away (Fig. 2).

The Vβ8.2 T cell receptor (TCR) component is believed to

be the predominant Vβ gene product associated with

antigen specific CD4+ T cell response to the major

encephalitogenic epitope of myelin basic protein (MBP) in

Lewis rats. Lewis rats were actively immunised with MBP,

5   and OX-40 positive and negative cells were analysed for

antigen reactivity and TCR Vβ utilisation (Fig 3 and 4).

Sorted OX-40⁺ T cells isolated from the spinal cord were

highly enriched for expression of the Vβ8.2 T cell

receptor component compared to the OX-40⁻ or unsorted

10   spinal cord populations (see Fig. 3). Figure 4 shows

that whereas both MBP and PPD reactive T cells showed

enriched responses in the OX-40⁺ fraction of the draining

lymph node, only MBP reactive T cells were found in the

OX-40⁺ fraction in the inflamed CNS. These data

15   demonstrate the selective ability of the OX-40 antibody

to mark the autoantigen reactive pathogenic T cells

within the affected target organ in EAE. These data

strongly suggest that Ag specific T cells can be isolated

.and characterised at the site of inflammation with the

20   OX-40 antibody, thereby diagnosing the cell type directly

involved with inflammation caused by autoantigen

recognition. The data also imply that isolation of OX-40⁺

cells will be useful in identifying Vβ biases and

autoantigen specific cells within inflamed tissues even

25   when the antigen specificity is unknown.

Therefore, the OX-40 antigen is shown to be expressed *ex

vivo* on antigen activated CD4⁺ T-cells and, furthermore,

this antigen is shown to be exclusively expressed on the
cells at inflammatory sites where the antigen is present
(OX-40 is not expressed on cells at non-inflammatory
sites in the absence of antigen). These results (the

5   transient nature and target organ expression of the OX-40
marker) suggested that OX-40 may be a diagnostic marker
and a suitable target for antibody mediated deletion of
activated autoimmune CD4⁺ T-cells.

The deletion of selective subsets of lymphocytes can
be mediated by antibodies or other binding proteins *in*

10   *vivo*. This can be done either by choosing an antibody or
other binding molecule which upon binding to cells
expressing OX-40 will then activate other effector cells
or proteins of the immune system to destroy the targetted

15   cells: examples of this include lysis of cells via
activation of the complement cascade or via triggering of
antibody-dependent cellular cytotoxicity (ADCC) (see Hale
et al 1983, Blood 62: 873-82: Greenwood et al 1993, Eur.
J. Immunology 23: 1098-1104); or by modifying the

20   antibody or binding molecule such that a toxic agent is
attached which will kill the cell upon binding and
ingestion.

It is possible to modify binding proteins, monoclonal

25   antibodies or fragments thereof by a variety of means.
For instance, it is possible by means of standard
molecular biological techniques to construct a cDNA
encoding a fusion protein part of which is a toxin (eg

see Williams et al 1987, Protein Engineering 1: 493-98).

Alternatively, one can couple toxins, drugs or other molecules to proteins by standard chemical coupling

5   procedures such as via thioether bonds (Glennie et al 1987, J. Immunology 139: 2367).

Several groups have shown that antibodies linked to toxic molecules (termed immunotoxins) can deplete cell

10   populations expressing the appropriate antigen (Fulton, R.J., et al. 1988). The advantage of immunotoxins is that they are highly selective in their target cell specificity and that small doses can eliminate unwanted/potentially harmful cells.

15

A variety of cytotoxins can be used to produce immunotoxins. Ricin A chain-antibody conjugates have been used to delete both normal and neoplastic lymphocytes in vivo and in vitro (Fulton, R.J. et al.,

20   1988; Street, N. E. et al., 1987). Other toxins such as Pseudomonas exotoxin A and diphtheria toxin have also been conjugated to antibodies and used to kill specific populations of cells (May, R.D., and Fulton, R.J. 1992).

25   In the late 1980s and early '90s several human Phase I/II clinical trials were performed using antibodies conjugated to the Ricin A chain (Weiner, L.M., et al.1989; Spliter, L.E., et al., 1987; Vitetta, E.S., et

al., 1991). Most of the trials have involved usi.g
antibodies specific for cancer antigens in order to lower
the tumor burden in cancer patients. Recently, there has
been a development of "second generation" immunotoxins

5   which have avoided some of the problems of non-specific
immunogenicity and toxicity in the treated patients.
This strategy uses the deglycosylated form of the Ricin A
(dgA) chain conjugated to the tumor specific antibody.
One such Phase I study used this modified form of the

10   immunotoxin against B cell lymphomas in 15 patients
(Vitetta, E.S., et. al., 1991). Approximately 40% of the
patients achieved partial remissions in which their
overall tumor burden was reduced by 50% or more. Killing
of the tumor cells was rapid occurring within 1 week

15   after completion of the therapy.

All of the *in vivo* studies presented herein use an OX-40
antibody-dgA conjugate. The antibody ricin A conjugation
was performed with a heterobifunctional cross-linker GPDP

20   or SMPT by the method described by May, R.D. and Fulton,
R.J. (1992). Briefly, a free amino group on the OX-40
antibody was reacted with the crosslinker and the
macromolecule was purified. The purified OX-40 antibody
product was then reacted with reduced ricin A chain

25   (which has one free cysteine) and the hybrid molecule was
purified.

Initially the rat OX-40 antibody was conjugated to the

dgA form of Ricin and this heteroconjugate was used to inhibit the *in vitro* proliferation of antigen specific CD4⁺ T-cell lines. The T-cell lines used were specific for MBP and upon adoptive transfer caused EAE in naive recipients. Fig. 5 shows that the OX-40 heteroconjugate inhibited antigen specific proliferation of the MBP specific T-cell line in a dose dependent manner with 50% inhibition at approximately 20 ng/well. A control using Ricin A alone showed inhibition of the assay only at high concentrations 500 ng/well and above, but no effect on the assay at lower concentrations (data not shown). Controls using the OX-40 anti-ody alone and an isotype-matched unrelated antibody conjugated to dgA also showed no inhibition (data not shown).

The OX-40 antibody was then conjugated to the Pseudomonas exotoxin and this conjugate was tested for inhibitory effect on antigen specific (MBP) CD4⁺ T-cell proliferation (Figures 6A-6B). This heteroconjugate was approximately 4-fold more efficient at inhibiting the *in vitro* assay, and the toxin alone did not inhibit the assay at any concentration. This assay was performed with a Lewis MBP specific line (Fig. 6B) and an F1 (Lewis x Buffalo) MBP specific line (Fig. 6A) with the same results, showing that the inhibition of proliferation was not strain specific

The OX-40-dgA was also used to inhibit the *in vitro*

proliferation of a CD4⁺ T-cell line activated with an antigen that was irrelevant to EAE or autoimmunity. The antigen used was the purified protein derivative (PPD) antigen from *Mycobacterium tuberculosis*. This antigen was used to ascertain whether the OX-40 antigen was specific to CD4⁺ T-cells activated by the MBP antigen, or whether it is present on the surface of all antigen activated CD4⁺ T-cells regardless of T-cell receptor specificity. As shown in Fig. 7, there was a dose dependent inhibition of proliferation of the PPD activated T-cell line with a comparable 50% inhibition to the MBP activated lines. This shows that the OX-40 immunotoxin will inhibit proliferation of any activated CD4⁺ T-cell line regardless of the antigen specificity.

Since the OX-40 immunotoxin was effective at inhibiting the proliferation of MBP specific autoimmune CD4⁺ T-cells in vitro, experiments were then performed to determine the potential of this immunotoxin to kill MBP specific CD4⁺ T-cells *in vivo*. To this end irradiated rats were used initially; these animals received an encephalitogenic dose of MBP specific F1 T-cells. At the same time the animals were injected with the OX-40-Ricin A conjugate or Ricin A alone. The effect of irradiation is to deplete the rat's immune system so that it would not recognize and deplete the allelic variant F1 donor T-cells. The use of irradiated rats facilitates the detection of the donor T-cells with the RT7.2 antibody in

the host after the transfer of these cells and allows the
fate of these transferred cells to be determined (see
Figs. 1, 2, 8A and 8B).

5   As shown in Table I, experiments 1 and 2, only 1/8
animals receiving the OX-40 immunotoxin showed clinical
signs of disease while all 8 animals that received
unconjugated Ricin A came down with EAE. The inhibitory
effect of the immunotoxin appeared to be mediated by the
10 OX-40 antibody since the Ricin A chain alone showed the
same disease score when compared to animals injected with
encephalitogenic CD4+ T-cells alone (data not shown).

Table I
15 OX-40 Ricin A Immunotoxin Effect on
Experimental Autoimmune Encephalomyelitis

Transfer Dose[a]	Treatment[b]	Incidence	Day of Onset	EAE Score[c]
Exp 1				
6.5 x 10^6	OX-40-Ricin A	1/3	6	0.66
6.5 x 10^6	Ricin A	3/3	4	6.33
Exp 2				
10 x 10^6	OX-40-Ricin A	0/5	--	--
10 x 10^6	Ricin A	5/5	5	4.90
Exp 3				
10 x 10^6	OX-40-Ricin A	0/2	--	--
10 x 10^6	No treatment	3/3	4	6.50

30 [a]MBP specific CD4+ T-cells were stimulated for 3 days *in vitro* with antigen and antigen presenting cells and transferred into naive recipients.
[b]400 μg of OX-40-Ricin A or the same molar amount of Ricin A alone was injected at the same time th; cells were
35 transferred.
[c]Value represents the mean cumulative EAE score for each group. 0, no signs; 1, limp tail; 2, hind leg weakness; 3, hind quarter paralysis; 4, moribund.

40 The data in Table I was generated using the EAE model

where F1 MBP T-cell lines (Lewis x Buffalo) were injected into irradiated Lewis (parental host) recipients. Thus, the transferred T-cell population could be detected in the spinal cord of these animals with the RT7.2 (Buffalo) antibody. Figs. 8A and 8B show the outcome of the OX-40-dgA treatment as assessed by the number of donor cells isolated from the spinal cord of the treated (clinically well) and control groups (paralyzed) on the first day of disease onset. A total of 200,000 spinal cord lymphocytes was isolated from the control group and 80% were found to be donor derived. In contrast 80,000 spinal cord lymphocytes was isolated from the treated group and only 15% were found to be donor derived. There was over a log-fold difference in the total amount of donor derived MBP reactive CD4$^+$ T-cells isolated from the spinal cord (the inflammatory site) between the two groups (160,000 vs. 12,000). This suggested that OX-40 dGA conjugate was specifically deleting the MBP-reactive CD4$^+$ T-cells in vivo.

As shown in Figure 9, animals were treated with OX-40 immunotoxin on the first day of disease onset when both groups had an average clinical score of 1. The day after treatment all the control animals showed signs of complete hind limb paralysis (score = 3), while the treated animals progressed only slightly (score = 1.5). The mean cumulative score after treatment was 8.0 in the control group and 3.75 in the treated group.

In a separate experiment, animals were treated on the
first day of disease, and were sacrificed 24 hr later.
The lymphocytes were then isolated from the spleens and
spinal cords. Three groups were analysed for in vivo

5   labelling of the OX-40 antibody. The controls received
no treatment, the second group received unconjugated OX-
40, and the third group received the OX-40 immunotoxin.
Similar number of cells were recovered from the spleens,
but half the amount of spinal cord lymphocytes were

10   recovered from the OX-40 immunotoxin-treated animals
compared to the control and the unconjugated OX-40-
treated groups. In vivo binding of the OX-40 antibody
was detected with an anti-mouse Ig-FITC. Approximately
15-20% of the lymphocytes isolated from the spinal cords

15   of OX-40 and OX-40 immunotoxin treated rats were anti-
mouse Ig positive. Cells isolated from the spinal cords
of control rats were mouse Ig negative, even though 18%
of the lymphocytes were OX-40 positive (Table Ia). There
were very few anti-mouse Ig-FITC positive cells isolated

20   from the spleens in any of the groups. A similar
percentage of OX-40$^+$ cells and anti-mouse Ig$^+$ cells were
isolated from the spinal cord of the OX-40 and OX-40
immunotoxin groups. The majority of anti-mouse Ig
positive cells were associated with the donor population

25   isolated from the spinal cord (RT7.2$^+$/anti-mouse Ig$^+$;
Table Ia). These results demonstrate that the in vivo
administration of the OX-40 antibody resulted in the
exclusive binding of autoantigen specific cells isolated

from the inflammed tissue.

TABLE Ia: In Vivo Labelling of OX-40 Positive T Cells

Treatment[a]			Donor[+]/
Cell Isolation mouse Ig[+]	OX-40[+]	α-mouse Ig[+]	Donor[+] α-
Control Spinal Cord 0.3%	17.9%[b]	0.2%	54.4%
Spleen 0.2%	0.5%	0.3%	2.4%
OX-40 Spinal Cord 13.5%	17.7%	17.5%[c]	53.2%
Spleen 0.3%	0.3%	0.4%	4.6%
OX-40-Ricin A[d] Spinal Cord 18.0%	19.0%	18.1%	50.3%
Spleen 0.4%	0.7%	0.5%	2.3%

[a] F1 T cells specific for MBP were transferred into irradiated Lewis hosts and 400 μg of OX-40 immunotoxin or OX-40 ab alone was administered i.p. the day of disease onset.
[b] All of the percent positive cells were determined by FAC's analysis using isotype control antibodies to draw quadrants for negative comparisons.
[c] The mean fluorescence intensity (MFI) of the α-mouse Ig^{+} cells was very similar to MFI of the OX-40 FITC stained cells.
[d] The total number of spinal cord lymphocytes isolated from the OX-40 immunotoxin animals was half that of the OX-40 and the control groups.

In order to assess the efficacy of the treatment we

isolated spinal cord lymphocytes and analysed the amount

of BP reactive donor cells with the RT7.2 antibody from

OX-40 immunotoxin treated rats versus control rats (F1

donor cells transferred into irradiated Lewis

recipients). Fig. 10A and 10B show the outcome of OX-40
immunotoxin administered on the same day of cell transfer
as assessed by the number of myelin reactive donor T
cells isolated from the spinal cord. RT7.2-positive
cells were analysed the first day of disease onset; the
treated animal was clinically well (EAE score = 0, with
1.2×10^4 donor$^+$ spinal cord lymphocytes) while the
control animal was paralysed (EAE score = 1, with 1.6 x
10^5 donor$^+$ spinal cord lymphocytes). In Figs. 10C, D, E
and F the treatment was given the day before disease
onset, when the only OX-40 positive donor T cells
isolated from the rats were from the spinal cord (Fig.
1). Spinal cord and spleen lymphocytes were isolated 24
hr after the treatment. The number of donor-derived
spleen cells isolated from the recipients was unaffected
by the treatment (data not shown). In all the
comparisons the number of donor$^+$ cells isolated from the
spinal cord was decreased by over a log in the treated
animals (Fig. 10). These data demonstrate that the OX-40
immunotoxin specifically depleted MBP reactive donor
cells in vivo, and as a consequence less encephalitogenic
cells were isolated from the spinal cord of the treated
animals.

The same in vivo experiments were then performed in a
non-irradiated host; a Lewis MBP activated T-cell line
was transferred into a Lewis host. The non-irradiated
host was used because this host has an intact immune

system more similar to human patients suffering from these same types of autoimmune diseases. In these experiments it was initially observed that a single injection of the conjugate at the time of transfer of

5 T-cells only partially inhibited subsequent development of the disease. Therefore experiments were performed utilizing two injections at different times.

Three animals were injected on day 0 and day 3 and

10 compared to 3 control animals (Table II). These results suggest that the immunotoxin was recognizing and killing the autoimmune T-cells at the site of inflammation on day 3 because, (1) the only donor T-cells expressing the OX-40 antigen on day 3 (the day before disease onset)

15 were in the spinal cord compartment (Fig. 1) and (2) the highest percentage of OX-40 expression on donor T-cells in the spinal cord was the day before disease onset (Fig. 2).

Table II
OX-40-Ricin A Immunotoxin Effect on Experimental
Autoimmune Encephalomyelitis (in non-irradiated host)

Transfer Dose[a]	Treatment[b]	Days of Injection	Day of Onset	EAE Score[c]
9.0 x 10^{6}	OX-40-Ricin A	0/3	5	1.33
9.0 x 10^{6}	Nothing	--	4	8.25

[a]Lewis MBP specific CD4^{+} T-cells were stimulated for 3 days *in vitro* with antigen and antigen presenting cells and transferred into Lewis recipients.

30 [b]400 μg of OX-40-Ricin A or the same molar amount of Ricin A alone was injected at the same time the cells were transferred.

[c]Value represents the mean cumulative EAE score for each

35 group of 3 animals. 0, no signs; 1, limp tail; 2, hind

leg weakness; 3, hind quarter paralysis; 4, moribund.

The conclusion from the data presented is that the OX-40 immunotoxin is extremely effective at killing/ inhibiting antigen specific CD4⁻ T-cell function both *in vitro* and *in vivo*. The effect of this specific immunotoxin does not seem to be restricted by strain or antigen specificity and will most likely have a wide range of applications *in vivo*.

The experiments described above were performed using the rat model system and antibodies against the rat OX-40 protein. However, the rat OX-40 antibody does not recognize human or murine activated CD4⁻ T-cells (data not shown). To facilitate the development of the present invention for human therapeutic use, it is necessary to produce antibodies against the human OX-40 protein.

To that end, a human cDNA encoding the human OX-40 homolog was cloned. Initially, two oligonucleotide primers were synthesized for use in the polymerase chain reaction (PCR). These primers were designed to amplify the full length OX-40 cDNA sequence; one primer was homologous to the coding strand in the region of the start codon of the rat cDNA sequence and the other was the inverse complement of the coding strand in the region of the stop codon of the rat cDNA sequence. Surprisingly, no product was ever obtained when these PCR primers were used with RNA isolated from activated CD4⁻ T

lymphocytes from humans.

Since the standard method of cloning the human OX-40 cDNA was unsuccessful, an alternative approach was required. First, the PCR primers were successfully used to clone the murine OX-40 cDNA by PCR from RNA isolated from murine CD4⁻ T-cells activated with Concanavalin A (data not shown). Then, the murine OX-40 cDNA was used to probe a cDNA lambda gt11 library from human activated T lymphocytes (No. HL10316 purchased from Clontech, Palo Alto, CA). Five similarly sized positive clones (1050-1200 bp) were obtained. These five recombinant lambda clones were subcloned into the Bluescript plasmid (Stratagene, La Jolla) and then sequenced on the 370A automated sequencer (Applied Biosystems, Pasadena CA). The sequence of the human OX-40 cDNA is set forth in SEQ. ID No. 1 in the accompanying sequence listing and is shown in Fig. 11.

A comparison of the predicted amino acid sequence of the human OX-40 protein with peptide sequences in the Genbank database indicated a high degree of homology with the murine OX-40 and rat OX-40 sequences; the probabilities that the predicted amino acid sequence of the human OX-40 protein shown in Seq. I.D. No. 1 was not related to the murine or rat OX-40 amino acid sequences were predicted to be 3.4×10^{-58} and 2.9×10^{-56} respectively. The next most closely related peptide sequence gave a probability

of 1.1 x 10⁻¹¹. Furthermore, a comparison of the
homologies between the human and rat OX-40 cDNA and amino
acid sequences over a 64 amino acid (192 base pair)
region starting at amino acid 31 of the rat sequence

5   revealed an amino acid homology of 62.5% and a nucleotide
homology of 67.5%. All ten cysteine residues within this
64 amino acid stretch were conserved.

OX-40 Expression in GVHD

10   The expression of OX-40 on T-cells was studied in
patients with graft versus host disease (GVHD).

Three patients who underwent allogeneic bone marrow
transplantation came down with GVHD. The day of GVHD
15   onset varied between the patients from 7-50 days after
transplant. Table 3 shows a summary of OX-40 expression
during the GVHD episode. All the patients showed an
increased percentage of CD4⁺ peripheral blood cells
expressing OX-40 during the early stage of disease
20   development. In all the patients the percentage of OX-40⁺
T cells declined after the initial clinical episode (7-14
days post GVHD). This data is consistent with the
transient expression of OX-40 observed during the early
stages of disease development in EAE (Fig. 2). OX-40
25   expression in GVHD correlated with the early development
of the clinical signs and may have direct diagnostic and
therapeutic applications for this and other human
transplant/autoimmune disorders.

Table 3

OX-40 Expression in PBLs of Bone Marrow Transplant
Patients with Graft Versus Host Disease

Patient	Days Post GVH	%CD4$^+$	%OX-40$^+$ of CD4[a]	Severity[b]
#1	3	5.8[c]	24.0[c]	grade 4
	7	5.0	0.5	
	14	4.0	1.6	
#2	1	12.1	10.5	grade 2
	10	6.6	0.5	
#3	2	41.0	10.0	grade 2
	8	8.3	0.6	

[a] Donor and recipient peripheral blood lymphocytes were
screened for OX-40 expression prior to the transplant
process and the CD4$^+$ T cells were less than 1% OX-40$^+$.

[b] Value represents the maximum severity each patient
obtained during clinical signs of GVHD (skin
involvement). 0, no signs; 1 - greater than no
involvement but involving < 25% of skin; 2 - > 25%
involvement but < 50% involvement; 3 - > 50% involvement;
4 - > 50% involvement with blisters; 5 - leading to
death.

[c] Lymphocytes from the peripheral blood of GVHD patients
were isolated by Hypaque-Ficoll. Samples were isolated
and analysed at various days post GVH. The samples were
dual stained with an anti-human CD4-PE antibody and an
anti-human OX-40 antibody. The OX-40 antibody was
detected with an anti-mouse IgG$_1$-FITC and the indirect
antibody alone was used as the negative control.

OX40 and IBD

It has been found that OX-40 can be of use in connection

with IBD.

The tissue expression of OX40 was investigated using the

standard technique of indirect alkaline phosphatase

immunohistochemical staining (see eg Immunocytochemistry:

Practical Applications in Pathology and Biology. J. Polak
and S. van Noorden, eds. John Wright and Sons, Bristol).
Biopsy tissue samples of intestine from both ulcerative
colitis and Crohn's disease patients stained positively

5   with an anti-OX40 antibody. Clusters of OX40+ cells were
seen among the lymphoid cells infiltrating the lamina
propria at sites of inflammation. In samples of
intestinal tissue from normals or in samples of
uninvolved intestinal tissue from patients only isolated,

10 scattered OX40+ cells were seen.

                            EXAMPLE ONE

Having herein provided the sequence of the human OX-40
cDNA, one skilled in the art will recognize that the full

15 length cDNA clone can now readily be obtained by standard
methods. Such methods include, for example, the
polymerase chain reaction (PCR) by which means DNA
sequences can be amplified. Methods and conditions for
PCR amplification of DNA are described in Innis et al.

20 (1990) and Sambrook et al. (1989).

The selection of PCR primers for amplification of the
human OX-40 cDNA will be made according to the portions
of the cDNA which are desired to be amplified. Primers

25 may be chosen to amplify small fragments of the cDNA or
the entire cDNA molecule. Variations in amplification
conditions may be required to accommodate primers of
differing lengths; such considerations are well known in

the art and are discussed in Innis et al. (1990). By way
of example only, the entire cDNA molecule corresponding
to the human OX-40 cDNA may be amplified using the
following primers. Primers 1 and 2 are also set forth in

5   the accompanying sequence listing as SEQ I.D. Nos. 2 and
3, respectively.

Primer 1:   5' ATGTGCGTGGGGGCTCGGCGGCTG 3'

Primer 2:   5' TCAGAACTTGACCAGGGTGGAGTG 3'

Template DNA for PCR amplification to produce the human

10   OX-40 cDNA can be extracted from the lambda GT11 cDNA
library from human activated T lymphocytes produced by
Clontech, Palo Alto, California (Catalog No. HL10316).

Alternatively, the human OX-40 cDNA may be obtained by

15   PCR amplification of reverse transcribed RNA (RT-PCR)
(Veres et al., 1987; Kawasaki et al., 1990).
Essentially, total RNA is extracted from activated human
CD4 T-cells by any one of a variety of methods routinely
used as described in Sambrook et al. (1989) and Ausubel

20   et al. (1987). Suitable human CD4 T-cells include the
human CD4^{+} T-cell lymphoma cell line described by
Gootenberg et al. (1981). Alternatively, activated CD4^{+}
T-cells can be isolated from human peripheral blood as
described by Kruisbeek (1992). The extracted RNA is then

25   used as a template for performing RT-PCR amplification of
the human OX-40 cDNA.

Standard methods for the purification and cloning of PCR

products are well known in the art and are described by
Innis et al. (1990) and Sambrook et al. (1989).

EXAMPLE TWO

With the provision of the human OX-40 cDNA, the
expression and purification of the human OX-40 protein by
standard laboratory techniques is now enabled. The
purified protein may be used for antibody production and
patient therapy.

Partial or full-length cDNA sequences, which encode for
the subject protein, may be ligated into bacterial
expression vectors. Methods for expressing large amounts
of protein from a cloned gene introduced into *Escherichia
coli* (*E. coli*) may be utilized for the purification of
the human OX-40 protein. For example, fusion proteins
consisting of amino terminal peptides encoded by a
portion of the *E. coli lacZ* or *trpE* gene linked to the
part or all of the human OX-40 protein may be used to
prepare polyclonal and monoclonal antibodies that
recognize the human OX-40 protein. Intact, native
proteins may also be produced in *E. coli* in large amounts
for functional studies. Methods and plasmid vectors for
producing fusion proteins and intact native proteins in
bacteria are described in Sambrook et al. (1989) (ch. 17,
herein incorporated by reference). Such fusion proteins
may be made in large amounts, are easy to purify, and can
be used to produce antibodies. Native proteins can be

produced in bacteria by placing a strong, regulated promoter and an efficient ribosome binding site upstream of the cloned gene. If low levels of protein are produced, additional steps may be taken to increase

5   protein production; if high levels of protein are produced, purification is relatively easy. Suitable methods are presented in Sambrook et al. (1989) and are well known in the art. Often, proteins expressed at high levels are found in insoluble inclusion bodies. Methods

10 for extracting proteins from these aggregates are described by Sambrook et al. (1989) (ch. 17). Vector systems suitable for the expression of lacZ fusion genes include the pUR series of vectors (Ruther and Muller-Hill, 1983), pEX1-3 (Stanley and Luzio, 1984) and pMR100

15 (Gray et al., 1982). Vectors suitable for the production of intact native proteins include pKC30 (Shimatake and Rosenberg, 1981), pKK177-3 (Amann and Brosius, 1985) and pET 3 (Studiar and Moffatt, 1986). Human OX-40 fusion proteins may be isolated from protein gels, lyophilized,

20 ground into a powder and used as antigen preparations.

For expression in mammalian cells, the cDNA sequence may be ligated to heterologous promoters, such as the simian virus (SV)40 promoter in the pSV2 vector (Mulligan and

25 Berg, 1981), and introduced into cells, such as monkey COS-1 cells (Gluzman, 1981), to achieve transient or long-term expression. The cDNA sequence (or portions derived from it) or a mini gene (a cDNA with an intron

and its own promoter) is introduced into eukaryotic expression vectors by conventional techniques. These vectors are designed to permit the transcription of the cDNA eukaryotic cells by providing regulatory sequences that initiate and enhance the transcription of the cDNA. Vectors containing the promoter and enhancer regions of the SV40 or long terminal repeat (LTR) of the Rous Sarcoma virus and polyadenylation and splicing signal from SV40 are readily available (Mulligan et al., 1981; Gorman et al., 1982). The level of expression of the cDNA can be manipulated with this type of vector, either by using promoters that have different activities (for example, the baculovirus pAC373 can express cDNAs at high levels in *S. frugiperda* cells (Summers and Smith, 1985)) or by using vectors that contain promoters amenable to modulation, for example, the glucocorticoid-responsive promoter from the mouse mammary tumor virus (Lee et al., 1982).

In addition, some vectors contain selectable markers such as the gpt (Mulligan and Berg, 1981) or neo (Southern and Berg, 1982) bacterial genes. These selectable markers permit selection of transfected cells that exhibit stable, long-term expression of the vectors (and therefore the cDNA). The vectors can be maintained in the cells as episomal, freely replicating entities by using regulatory elements of viruses such as papilloma (Sarver et al., 1981) or Epstein-Barr (Sugden et al.,

1985). Alternatively, one can also produce cell lines
that have integrated the vector into genomic DNA. Both
of these types of cell lines produce the gene product on
a continuous basis. One can also produce cell lines that
5   have amplified the number of copies of the vector (and
therefore of the cDNA as well) to create cell lines that
can produce high levels of the gene product (Alt et al.,
1978).

10   The transfer of DNA into eukaryotic, in particular human
or other mammalian cells, is now a conventional
technique. The vectors are introduced into the recipient
cells as pure DNA (transfection) by, for example,
precipitation with calcium phosphate (Graham and Vander
15   Eb, 1973) or strontium phosphate (Brash et al., 1987),
electroporation (Neumann et al., 1982), lipofection
(Felgner et al., 1987), DEAE dextran (McCuthan et al.,
1968), microinjection (Mueller.et al., 1978), protoplast
fusion (Schafner, 1980), or pellet guns (Klein et al.,
20   1987). Alternatively, the cDNA can be introduced by
infection with virus vectors. Systems are developed that
use, for example, retroviruses (Bernstein et al., 1985),
adenoviruses (Ahmad et al., 1986), or Herpes virus
(Spaete et al., 1982).

25

The human OX-40 protein expressed in eukaryotic cells may
be purified and used to produce antibodies. The human
OX-40 protein may be extracted following release of the

protein into the supernatant, or, the cDNA sequence may be incorporated into a eukaryotic expression vector and expressed as a chimeric protein with, for example, β-globin. Antibody to β-globin is thereafter used to

5   purify the chimeric protein. Corresponding protease cleavage sites engineered between the β-globin gene and the cDNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating β-globin chimeric

10   proteins is pSG5 (Stratagene, La Jolla, CA). This vector encodes rabbit β-globin.

This invention encompasses recombinant cloning vectors containing the human OX-40 cDNA sequence, or portions

15   thereof. The human OX-40 cDNA is operatively linked in the vector to an expression control sequence in the recombinant DNA molecule so that the human OX-40 polypeptide, or a portion thereof, can be expressed. The expression control sequence may be selected from the

20   group consisting of sequences that control the expression of genes of prokaryotic or eukaryotic cells and their viruses and combinations thereof. The expression control sequence may be specifically selected from the group consisting of the *lac* system, the *trp* system, the *tac*

25   system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the early and late promoters of SV40, promoters derived from polyoma, adenovirus, retrovirus, baculovirus

and simian virus, the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, the promoter of the yeast alpha-mating factors and combinations thereof.

5

The host cell, which may be transfected with the vector of this invention, may be selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus subtilis*, *Bacillus·stearothermophilus* or other bacilli; other bacteria; yeast; fungi; insect; mouse or other tissue cells, including human tissue culture cells.

In a preferred embodiment of the present invention, the full length human OX-40 cDNA as shown in Fig. 9 (from start codon to stop codon) is ligated into a baculovirus vector and the recombinant human protein is produced in the appropriate insect cells. Suitable baculovirus expression systems include the BacPAK™ Baculovirus Expression System produced by Clontech (Palo Alto, CA).

20 Thus, by way of example, the full length human OX-40 cDNA is ligated into the plasmid pBacPAK1 and expressed in *Spodoptera fugiperda* cells according to the manufacturer's instructions.

25 The human OX-40 protein produced in the insect cells is then purified by standard techniques. A preferred technique of isolating the recombinant product is to use a vector that adds an additional 6 residues of histidine

to the recombinant protein. Fusion proteins produced in this manner chelate metal, which facilitates protein purification enormously. Thus, for example, in high salt, polyhistidine fusion proteins bind with a high

5   affinity to a metal chelate matrix whereas the majority of host proteins do not bind at all. Low affinity binding host proteins can be washed off the matrix by decreasing the pH to 6.0. Specific elution of the polyhistidine-containing fusion protein can be

10   accomplished with 300 mM imidazole buffer at pH 6.0.

                        EXAMPLE THREE

Monoclonal antibodies may be produced to the human OX-40 protein for therapeutic use. Substantially pure human

15   OX-40 protein suitable for use as an immunogen is isolated from the transfected or transformed cells as described in Example 2 above. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of

20   a few milligrams per millilitre. Monoclonal antibody to the protein can then be prepared as follows:

        A. Monoclonal Antibody Production by Hybridoma
Fusion.

25   Monoclonal antibody to epitopes of the human OX-40 protein identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler and Milstein (1975) or

derivative methods thereof. Briefly, a mouse is
repetitively inoculated with a few micrograms of the
selected purified protein over a period of a few weeks.
The mouse is then sacrificed, and the antibody-producing
cells of the spleen isolated. The spleen cells are fused
by means of polyethylene glycol with mouse myeloma cells,
and the excess unfused cells destroyed by growth of the
system on selective media comprising aminopterin (HAT
media). The successfully fused cells are diluted and
aliquots of the dilution placed in wells of a microtiter
plate where growth of the culture is continued.
Antibody-producing clones are identified by detection of
antibody in the supernatant fluid of the wells by
immunoassay procedures, such as ELISA, as originally
described by Engvall (1980), and derivative methods
thereof. Selected positive clones can be expanded and
their monoclonal antibody product harvested for use.
Detailed procedures for monoclonal antibody production
are described in Harlow and Lane (1988).

B. Antibodies Raised Against Synthetic Peptides.
An alternative approach to raising antibodies against the
human OX-40 protein is to use synthetic peptides
synthesized on a commercially available peptide
synthesizer based upon the predicted amino acid sequence
of the human OX-40 protein shown in Figure 11.

In a preferred embodiment of the present invention,

monoclonal antibodies that recognize the human OX-40

protein are produced. Optimally, monoclonal antibodies

raised against the human OX-40 protein specifically

detect the human OX-40 protein. That is, such antibodies

5   recognize and bind the human OX-40 protein and do not

substantially recognize or bind to other proteins found

in human cells. Put another way, such antibodies have a

specificity of binding in humans to substantially only

the human OX-40 protein and thus to substantially only

10   activated CD4$^+$ T-cells.   .

The determination that an antibody specifically detects

the human OX-40 protein is made by any one of a number of

standard immunoassay methods; for instance, the Western

15   blotting technique (Sambrook et al., 1989). To determine

that a given antibody preparation (such as one produced

in a mouse) specifically detects the human OX-40 protein

by Western blotting, total cellular protein is extracted

from human cells that do not express the OX-40 antigen,

20   such as non-activated lymphocytes. As a positive

control, total cellular protein is also extracted from

activated T-cells especially for example activated CD4$^+$ T-

cells. These protein preparations are then

electrophoresed on a sodium dodecyl sulfate-

25   polyacrylamide gel. Thereafter, the proteins are

transferred to a membrane (for example, nitrocellulose)

by Western blotting, and the antibody preparation is

incubated with the membrane. After washing the membrane

to remove non-specifically bound antibodies, the presence

of specifically bound antibodies is detected by the use

of an anti-mouse antibody conjugated to an enzyme such as

alkaline phosphatase; application of the substrate 5-

5   bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium

results in the production of a dense blue compound by

immuno-localized alkaline phosphatase. Antibodies which

specifically detect the human OX-40 protein will, by this

technique, be shown to bind to the human OX-40 protein

10   band (which will be localized at a given position on the

gel determined by its molecular weight). Non-specific

binding of the antibody to other proteins may occur and

may be detectable as a weak signal on the Western blot.

The non-specific nature of this binding will be

15   recognized by one skilled in the art by the weak signal

obtained on the Western blot relative to the strong

primary signal arising from the specific antibody-human

OX-40 protein binding. Preferably, no antibody would be

found to bind to proteins extracted from the unactivated

20   T-cells especially for example CD4⁺ T-cells.

In addition to binding assays using extracted proteins,

monoclonal antibodies raised against the human OX-40

protein are tested to confirm their ability to recognize

25   the appropriate cell type (activated human T-cells

especially for example activated CD4⁺ T-cells) by

conjugating the human OX-40 antibody to a fluorescent tag

(such as FITC) and analyzing cell populations by FACS as

described above. The human OX-40 antibody will

preferably recognize activated T-cells especially for

example activated CD4⁺ T-cells. Therefore, dual staining

of an activated T-cell population with CD4-PE and OX-40-

5   FITC should show cells that are double positive.

Monoclonal antibodies for use in the present invention

will generally be of the IgM or IgG ir ·ype, and will

preferably be of mouse, human or other mammalian origin.

10

In one pref-rred embodiment of the present invention, the

monoclonal antibodies that recognize the human OX-40

antigen are mouse monoclonal antibodies that have been

"humanized". Such humanized antibodies can be more

15   safely administered to human patients than can unmodified

monoclonal antibodies produced in mouse cells.

Monoclonal antibodies produced in non-human cells, such

as mouse cells, generally evoke an immune response when   .

administered to a human host, thus limiting the duration

20   of the biological efficacy of the monoclonal antibody

(see generally, U.S. Patent No. 4,731,244 and WO

89/06976). Humanized antibodies are produced by

recombinant DNA technology and generally comprise the

antibody constant region from human monoclonal antibodies

25   combined with the variable (antigen recognition) region

from the mouse monoclonal antibody that recognizes the

target antigen (in this case the human OX-40 protein).

Because only the variable region is of murine origin,

humanized monoclonal antibodies are significantly less
likely to induce an immune response when administered to
a human patient.

5   Methods for humanizing antibodies are described by
Riechmann et al. (1988). Riechmann et al. introduced the
six hypervariable regions from the heavy and light chain
domains of a rat antibody into a human IgG1 antibody
directed against human lymphocytes. Riechmann et al.
10   showed that this "humanized" antibody was able to bind to
its target antigen *in vivo* without eliciting an anti-
immunoglobulin immune response.

For the preferred embodiments of this invention, intact
15   monoclonal antibodies are used. However, one skilled in
the art will recognize that portions of monoclonal
antibodies that are capable of recognizing and binding to
the human OX-40 protein may also be employed. These
antibody fragments generally include Fab, F(ab)'2 and Fv
20   fragments of antibodies which recognize the human OX-40
protein. Immunotoxins comprising antibody fragments have
been shown to be effective in deleting CD4$^+$ T-cells both
in vivo and *in vitro* using an antibody that recognizes
all cells that express the CD4 antigen (Street et al.,
25   1987).

EXAMPLE FOUR

As set forth in the preceding examples, this invention
enables the production of monoclonal antibodies that, in

humans, bind substantially only the human OX-40 protein. Such monoclonal antibodies can be used in therapeutic applications, for example in the form of conjugates with cytotoxic molecules, e.g. as antibody-ricin-A conjugate as discussed below. Such conjugates are commonly referred to as immunotoxins. Immunotoxins are characterized by two components; a cytotoxic agent which is usua..ly fatal to a cell when attached or absorbed, and a "delivery vehicle" which serves to deliver the cytotoxin to the target cell type. For the present invention, the target cell type is activated T-cells especially for example activated CD4$^+$ T-cells and the delivery vehicle is an antibody or antibody fragment that recognizes and binds to the human OX-40 antigen, as described in Example 8 below.

A number of suitable cytotoxins are known in the art, including: cytotoxic proteins such as the Ricin A chain, tne deglycosylated form of the Ricin A chain (dgA), the Pseudomonas exotoxin; radionuclides such as Iodine-131, Yttrium-90, Rhenium-188 and Bismuth-212; and a number of chemotherapeutic drugs such as vindesine, methotrexate, adriamycin and cis-platinum. (See generally, Olsnes and Phil (1982), and Baldwin and Byers (1985)). In one embodiment of the present invention, the cytotoxin is the deglycosylated form of the Ricin A chain, as described in U.S. Patent No. 4,590,071.

A selected cytotoxin may be conjugated with an anti-

human OX-40 antibody to produce an immunotoxin for use in the present invention. Antibodies may be conjugated with cytotoxins by a number of well known procedures, as generally described in Thorpe et al. (1982). For example, where the cytotoxic agent is a protein (such as the Ricin A chain) and the delivery vehicle is an intact monoclonal antibody, the linkage may be by way of heterobifunctional cross linkers, such as carbodiimide or gluteraldehyde. Preferred methods of producing immunotoxins using the deglycosylated Ricin A chain are provided in U.S. Patent No. 4,590,071, and WO 89/06967, which are herein incorporated by reference.

Immunotoxins as provided by the present invention and produced as described above are subsequently tested to confirm their *in vitro* efficacy. Such *in vitro* testing is performed using human CD4$^+$ T-cells and the methods described above. For example, an immunotoxin produced according to the present invention (i.e. a cytotoxin conjugated to a monoclonal antibody that has been shown to be specific to the human OX-40 protein), is tested using *in vitro* inhibition studies on MBP specific CD4$^+$ T-cell lines from multiple sclerosis patients. Immunotoxins potentially suitable for use in human therapy are those capable of inhibiting the *in vitro* proliferation of such cells.

Since these immunotoxins are capable of inhibiting the *in*

vitro proliferation of activated CD4⁺ T-cells from
multiple sclerosis patients, they should be capable of
inhibiting the proliferation of all activated CD4⁺ T-
cells, regardless of origin. This conclusion is
supported by the evidence set forth above, where the rat
Ox-40-dgA immunotoxin was shown to be effective against
MPB activated rat CD4⁺ T-cells and PPD activated rat CD4⁺
T-cells. To confirm that the human OX-40 immunotoxin has
this general activity, similar *in vitro* proliferation
studies as described above may also be performed with
human CD4⁺ T-cells specific for other antigens (such as
herpes simplex virus.)

In an alternative embodiment of the present invention
described in Example 5 below, anti-human OX-40 antibodies
can also be used to diagnose conditions mediated by
activated T-cells especially for example activated CD4⁺ T-
cells. For such applications, it is preferable that the
antibody is conjugated to a suitable chemical "tag" which
facilitates detection of the antibody. Suitable
molecules include the fluorescent molecules fluorescein
isothiocyanate (FITC) and R-phycoerythrin (PE) as
utilized in the present invention

EXAMPLE FIVE

In one embodiment of the present invention, monoclonal
antibodies that specifically bind the human OX-40 protein
are used to detect conditions mediated by activated T-

cells especially for example activated CD4⁺ T-cells. For

such purposes, human OX-40 antibodies are conjugated with

other molecules, such as fluorescent markers.

5   Biopsy samples are taken from inflamed tissue for

analysis. One skilled in the art will recognize that the

source of the biopsy sample will vary between different

conditions. In the case of multiple sclerosis the

lymphocytes will be isolated from the CSF, while in

10   rheumatoid arthritis the lymphocytes will be isolated

from the syncvial fluid of inflamed joints. In the case

of transplant rejection biopsies will be taken directly

from the target organ during a rejection episode.

15   In a preferred embodiment, a biopsy sample taken from a

patient will be fractioned into a lymphocyte fraction (by

methods described earlier; see Materials and Methods).

·The purified lymphocytes will be stained with the OX-40

FLTC antibody and the percentage of positive lymphocytes

20   will be quantitated on a FACScan apparatus. This

percentage will be compared with the percentage found in

healthy individuals. Any statistically significant

increase will provide an early indication of an

inflammatory event and will lead to early diagnosis of

25   autoimmune disorders.

EXAMPLE SIX

For therapeutic applications, such as treatment of autoimmune inflammations associated with multiple sclerosis, it is anticipated that the presence of

5   activated T-cells especially for example activated CD4$^+$ T-cells at the site of inflammation will be established before treatment is commenced. The presence of these cells can be established using the diagnostic methods described in Example 5 above. If the diagnostic test

10   produces a result indicating the presence of activated T-cells especially for example activated CD4$^+$ T-cells at the inflammatory site, then therapeutic application of the immunotoxin may be appropriate.

15   For therapeutic administration of the immunotoxins for treatment of conditions mediated by activated T-cells, especially for example activated CD4$^+$ T-cells, standard published protocols that set forth treatment regimes using immunotoxins may be utilized. These include

20   protocols described by Vitetta et al., 1991, and in WO89/06967. These documents are herein incorporated by reference.

In general, the method of treating a patient suffering

25   from a condition mediated by antigen-activated T-cells especially for example activated CD4$^+$ T-cells will comprise administering to the patient an effective amount of an antibody (or a portion of an antibody) conjugated

with a cytotoxic agent wherein the antibody (or the

portion of the antibody) recognizes and binds to the

human OX-40 antigen. As discussed above, antibodies and

portions of antibodies conjugated with a cytotoxic agent

5   are commonly referred to as immunotoxins. Effective

amounts of these immunotoxins may generally be referred

to as a suitable dose of an immunotoxin.

One skilled in the art will recognize that _any_ dose of

10   the immunotoxins greater than zero will have some effect

on the activated CD4$^+$ T-cell population in a patient.

However, suitable doses are limited by the onset of

adverse side effects of high doses of immunotoxin. As

described in WO89/06967, for immunotoxins comprising a

monoclonal antibody conjugated with the ricin A chain,

15   suitable doses are in the range of 0.05 - 1.0 mg/kg daily

for up to 14 days. As described by Vitetta et al.

(1991), for immunotoxins comprising antibody fragments

(such as the Fab' fragment) linked to the chemically .

20   deglycosylated ricin A chain, doses will preferably be in

the range of 25 - 150 mg/m^2.

EXAMPLE SEVEN

One embodiment of the present invention is a kit

25   containing monoclonal antibodies that recognize the human

OX-40 antigen. Such a kit would comprise a container

within which the monoclonal antibody is contained.

In one embodiment of such a kit, the kit would contain

the monoclonal antibody in a form conjugated with a

cytotoxin, such as dgA, whereby the kit could be used to

treat patients suffering from a condition mediated by

5   activated T-cells especially for example activated CD4⁺

T-cells. This antibody-cytotoxin conjugate would

preferably be provided in a form suitable for adminis-

tration to a patient by injection. Thus, the kit might

contain the antibody-cytotoxin conjugate in a suspended

10   form, such as suspended in a suitable pharmaceutical

excipient. Alternatively, the conjugate could be in a

solid form suitable for reconstitution.

In an alternative embodiment, the kit would contain the

15   monoclonal antibody in a form suitable for diagnostic

use, such as conjugated to a fluorescent marker. Such

kits would be used in the detection of inflammatory

conditions mediated by activated T-cells especially for

example activated CD4⁺ T-cells..

20

The foregoing examples are illustrative of the present

invention, but are not limiting. Numerous variations and

modifications on the invention as set forth can be

effected without departing from the spirit and scope of

25   the present invention.

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SEQUENCE LISTING

    (1)   GENERAL INFORMATION

   (i)   APPLICANTS: Andrew D. Weinberg

   (ii)   TITLE OF INVENTION: TREATMENT OF CD4 T-CELL
                                    MEDIATED CONDITIONS

  (iii) NUMBER OF SEQUENCES:. 3

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   (v)   COMPUTER READABLE FORM:

       (A) MEDIUM TYPE: Disk, 3-1/2 inch

       (B) COMPUTER: IBM PC compatible

       (C) OPERATING SYSTEM: MS DOS

       (D) SOFTWARE: WordPerfect 5.1

   (vi)   CURRENT APPLICATION DATA:

       (A) APPLICATION NUMBER:

       (B) FILING DATE:

       (C) CLASSIFICATION:

  (vii) PRIOR APPLICATION DATA: N/A

       (A) APPLICATION NUMBER:

       (B) FILING DATE:

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       (C) REFERENCE/DOCKET NUMBER: 4282-38649

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       (A) TELEPHONE: (503) 226-7391

       (B) TELEFAX: (503) 228-9446

(2)   INFORMATION FOR SEQ ID NO: 1

(i)   SEQUENCE CHARACTERISTICS:

    (A)   LENGTH: 848 base pairs

    (B)   TYPE: Nucleic acid

    (C)   STRANDEDNESS: Double

    (D)   TOPOLOGY: Linear

(ii)   MOLECULE TYPE: cDNA to mRNA

(iii)   HYPOTHETICAL: No

(iv)   ANTI-SENSE: No

(v)   FRAGMENT TYPE:

(xi)   SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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                                       CGGC AGAGACGAGG   14

ATG TGC GTC GGG GCT CGG CGG CTG GGC CGC GGG CCG TGT GCG GCT   59
met cys val gly ala arg arg leu gly arg gly pro cys ala ala
1               5                   10                  15

CTG CTC CTC CTG GGC CTG GGG CTG AGC ACC GTG ACG GGG CTC CAC   104
leu leu leu leu gly leu gly leu ser thr val thr gly leu his
            20                  25                  30

TGT GTC GGG GAC ACC TAC CCC AGC AAC GAC CGG TGC TGC CAC GAG   149
cys val gly asp thr tyr pro ser asn asp arg cys cys his glu
        35                  40                  45

TGC AGG CCA GGC AAC GGG ATG GTG AGC CGC TGC AGC CGC TCC CAG   194
cys arg pro gly asn gly met val ser arg cys ser arg ser gln
        50                  55                  60

AAC ACG GTG TGC CGT CCG TGC GGG CCG GGC TTC TAC AAC GAC GTG   239
asn thr val cys arg pro cys gly pro gly phe tyr asn asp val
            65                  70                  75

GTC AGC TCC AAG CCG TGC AAG CCC TGC ACG TGG TGT AAC CTC AGA   284
val ser ser lys pro cys lys pro cys thr trp cys asn leu arg
            80                  85                  90

AGT GGG AGT GAA CGG AAG CAG CTA TGC ACG GCC ACA CAG GAC ACA   329
ser gly ser glu arg lys gln leu cys thr ala thr gln asp thr
                95                  100                 105

GTC TGT CGC TGC CGG GCG GGC ACC CAG TCC CTG GAC AGC TAC AAG   374
val cys arg cys arg ala gly thr gln ser leu asp ser tyr lys
                110                 115                 120

CCT GGA GTT GAC TGT GCC CCC TCC CCT CCA GGG CAC TTC TCC CCA   419
pro gly val asp cys ala pro cys pro pro gly his phe ser pro
                125                 130                 135

GGC GAC AAC CAG GCC TGC AAG CCC TGG ACC ACC TGT ACC TTG GTT   464
gly asp asn gln ala cys lys pro trp thr thr cys thr leu val
                140                 145                 150

GGG AAG CAC ACC CTG CAG CCG GCC AGT AAT AGC TCG GAC GCA ATC   509
gly lys his thr leu gln pro ala ser asn ser ser asp ala ile
                155                 160                 165
```

```
    TGT GAG GAC AGG GAC CCC CCA GCC ACG CAG CCC GAG GAG ACC CAG    554
    cys glu asp arg asp pro pro ala thr gln pro gln glu thr gln
                170                 175                 180

5   GGT CCC CCG GCC AGG CCC ATC ACT GTC CAG CCC ACT GAA GCC TGG    599
    gly pro pro ala arg pro ile thr val gln pro thr glu ala trp
                185                 190                 195

10  CCC AGA ACC TCA CAG GGA CCC TCC ACC CGG TCC GTG GAG GTC CCC    644
    pro arg thr ser gln gly pro ser thr arg ser val glu val pro
                200                 205                 210

15  GGG GGC CGT GCG GTT GCC GCC ATC CTG GGA CTG GGA CTG GTG CTG    689
    gly gly arg ala val ala ala ile leu gly leu gly leu val leu
                215                 220                 225

    GGG CTG CTG GGA CCC CTG GAC ATC CTG CTG GCC CTG TAC CTG ATC    734
    gly leu leu gly pro leu asp ile leu leu ala leu tyr leu ile
                230                 235                 240
20

    CGG AGG CAC CAG AGG CTG CCC CCC GAT GCC CAC AAG CCC CCT GGG    779
    arg arg asp gln arg leu pro pro asp ala his lys pro pro gly
                245                 250                 255

25  GGA GGT AGC TTC CGG ACC CCC ATC CAA GAG GAG CAG GCC GAC GCC    824
    gly gly ser phe arg thr pro ile gln glu glu gln ala asp ala
                260                 265                 270

    CAC TCC ACC CTG GTC AAG TTC TGA                                848
30  his ser thr leu val lys phe
                275
35
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(3)   INFORMATION FOR SEQ ID NO: 2

(i)   SEQUENCE CHARACTERISTICS:

    (A)   LENGTH: 24 base pairs

    (B)   TYPE: Nucleic acid

5   (C)   STRANDEDNESS: Single

    (D)   TOPOLOGY: Linear

(iii) HYPOTHETICAL: No

(iv)   ANTI-SENSE: No

(xi)   SEQUENCE DESCRIPTION: SEQ ID NO: 2:

10   ATGTGCGTGG GGGCTCGGGC GGCTG   24

(4)   INFORMATION FOR SEQ ID NO: 3

(i)   SEQUENCE CHARACTERISTICS:

15   (A)   LENGTH: 24 base pairs

    (B)   TYPE: Nucleic acid

    (C)   STRANDEDNESS: Single

    (D)   TOPOLOGY: Linear

(iii) HYPOTHETICAL: No

20   (iv)   ANTI-SENSE: No

(xi)   SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TCAGAACTTG ACCAGGGTGG AGTG   24

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method of detecting a condition mediated by activated T-cells, eg CD4⁻ T-cells, in a patient, comprising contacting a specific binding agent with said T-cells and quantitating

5 the level of activated T-cells, wherein said specific binding agent is a specific binding partner able to bind a polypeptide having an amino acid sequence encoded by the nucleic acid sequence shown in SEQ ID No 1 or by bases 15-848 thereof or other sub-sequence thereof which encodes an

10 antigenic determinant, or a variant or allele or derivative thereof.

2. A method according to claim 1 wherein the specific binding agent is a specific binding partner able to bind a

15 polypeptide other than a protein consisting of the full sequence of human gp34 protein as defined in S. Miura et al. Mol. Cell Biol. 11(3)(1991) pp 1313-1325.

3. A method according to claim 2 wherein said specific

20 binding agent specifically binds to human Ox-40 of amino acid sequence encoded by the coding region of nucleic acid sequence Seq ID No 1, when said Ox-40 is present on the surface of activated T-cells.

25 4. A method according to any one of claims 1 to 3 wherein said specific binding agent comprises an antibody binding domain.

5. A method according to claim 4 wherein said specific

30 binding agent comprises a monoclonal antibody or a binding

fragment thereof.

6.   A method according to claim 5 wherein the specific binding agent is at least partially humanised.

5

7.   A method according to claim 6 wherein the specific binding agent comprises a humanised monoclonal antibody.

8.   A method according to any one of claims 1 to 7 wherein
10   the specific binding agent further comprises a molecularly linked cytotoxin.

9.   A method according to claim 8 wherein the specific binding agent comprises a Fab, F(ab')$_2$ or Fv fragment of a
15   monoclonal antibody conjugated with a cytotoxic agent.

10.   A method according to any one of claims 1 to 9 wherein the specific binding agent further comprises a label.

20   11.   A method according to claim 10 wherein the label is a radioactive label.

12.   A method according to claim 10 wherein the label is a fluorescent label.

25

13.   A method according to claim 1, wherein said specific binding agent comprises a monoclonal antibody, eg an antibody carrying a label for detection or quantitation, that binds substantially only to human OX-40 antigen.

30

14. A method according to any one of claims 1 to 13 which is carried out on a biopsy sample from the patient.

15. A method according to claim 14 wherein the biopsy
5 sample is from a patient suspected of having an inflammatory condition for detection of said condition.

16. A method according to claim 15 wherein the biopsy sample is e.g. a skin or intestinal biopsy sample or a blood
10 sample from a patient suspected of having a graft-versus-host disease.

17. A method according to claim 15 wherein the biopsy sample, e.g. an intestinal biopsy sample, is from a patient
15 suspected of having an inflammatory bowel disorder.

18. A method according to claim 14 wherein the biopsy sample is a sample of cerebrospinal fluid.

20 19. A kit for detecting a condition mediated by activated T-cells e.g. CD4' T-cells in a patient comprising a specific binding agent as defined in claim 10 or claim 11 or claim 12.

25 20. A kit for carrying out a specific binding assay for detection or quantitation of an analyte that comprises a polypeptide as defined in claim 1 or claim 2 or a specific binding agent as defined in claim 1 or claim 2, wherein said

kit comprises a first reagent comprising a specific binding agent that can recognise the analyte, a second reagent comprising a substance that can bind specifically either to the analyte or to the first reagent, and a label for the

5   second reagent.

21.   A kit according to claim 20, wherein the first reagent comprises an antibody specific for the analyte, and the second reagent comprises a labelled antiglobulin specific

10   for the first reagent.

22.   A kit according to claim 20, wherein the first reagent comprises an immobilised specific binding reagent for the analyte, the second reagent is a specific binding agent that

15   can bind to the analyte when the analyte is also bound to the first reagent.

23.   A kit according to claim 20, wherein the second reagent comprises a substance able to compete with analyte for

20   binding to the first reagent.

24.   A kit for treating a condition mediated by activated T-cells, eg activated CD4$^+$ T cells, said kit comprising a container containing a suitable amount of a specific binding

25   agent antibody according to any of claims 2 to 13 in a pharmaceutically acceptable excipient.

25.   Use of polypeptides as defined in claim 1 or claim 2

and/or specific binding agents as defined in any one of claims 1 to 13 in specific binding reactions for detection or quantitation of polypeptides as defined in claim 1 or claim 2 and/or specific binding agents complementary

5 thereto.

DATED this 4th day of November, 1998
Andrew Dale Weinberg AND Arthur Alan Vandenbark
By DAVIES COLLISON CAVE
Patent Attorneys for the Applicants

ABSTRACT

The OX-40 antigen is characterised and claimed together with variants and derivatives thereof. Also described are binding agents for the antigen and the use of these in diagnosis and therapy.

Examples of such use include a method for the selective depletion of activated CD4⁺ T-cells *in vivo* by using immunotoxins comprising an OX-40 antibody conjugated to a toxic molecule (such as Ricin-A chain). The administration of these specific immunotoxins is used therapeutically to deplete autoimmune reactive CD4⁺ T-cells which have been implicated in diseases including Multiple Sclerosis, Rheumatoid Arthritis, Sarcoidosis, and Autoimmune Uveitis as well as inflammatory bowel disease and graft-versus-host disease. This type of therapy is also beneficial for eradicating CD4⁺ T-cell lymphomas and alloreactive CD4⁺ T-cells involved with a transplantation reaction. The use of the human form of the OX-40 antibody will also help in the early diagnosis of all the diseases mentioned above.

Fig.1.
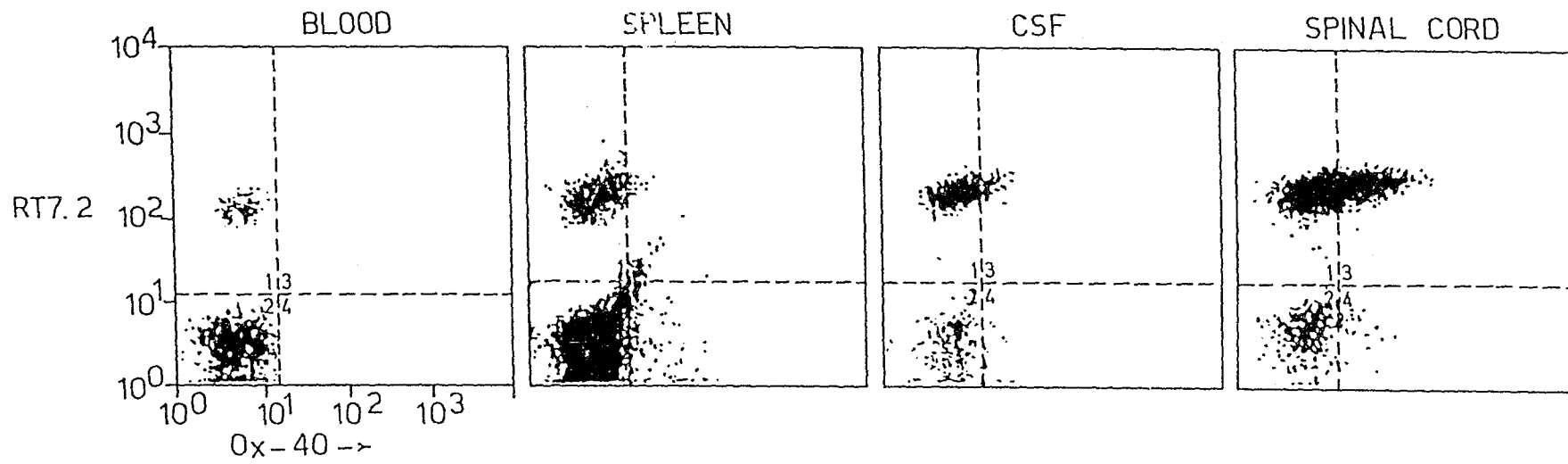
RT7.2 vs OX-40

TIME COURSE OF SPINAL CORD OX-40 EXPRESSION

Fig.2.

DAY 0 (EAE=0)

DAY 1 (EAE=1)

DAY 2 (EAE=2+)

DAY 3 (EAE=2)

DAY 5 (EAE=+)

RT7.2
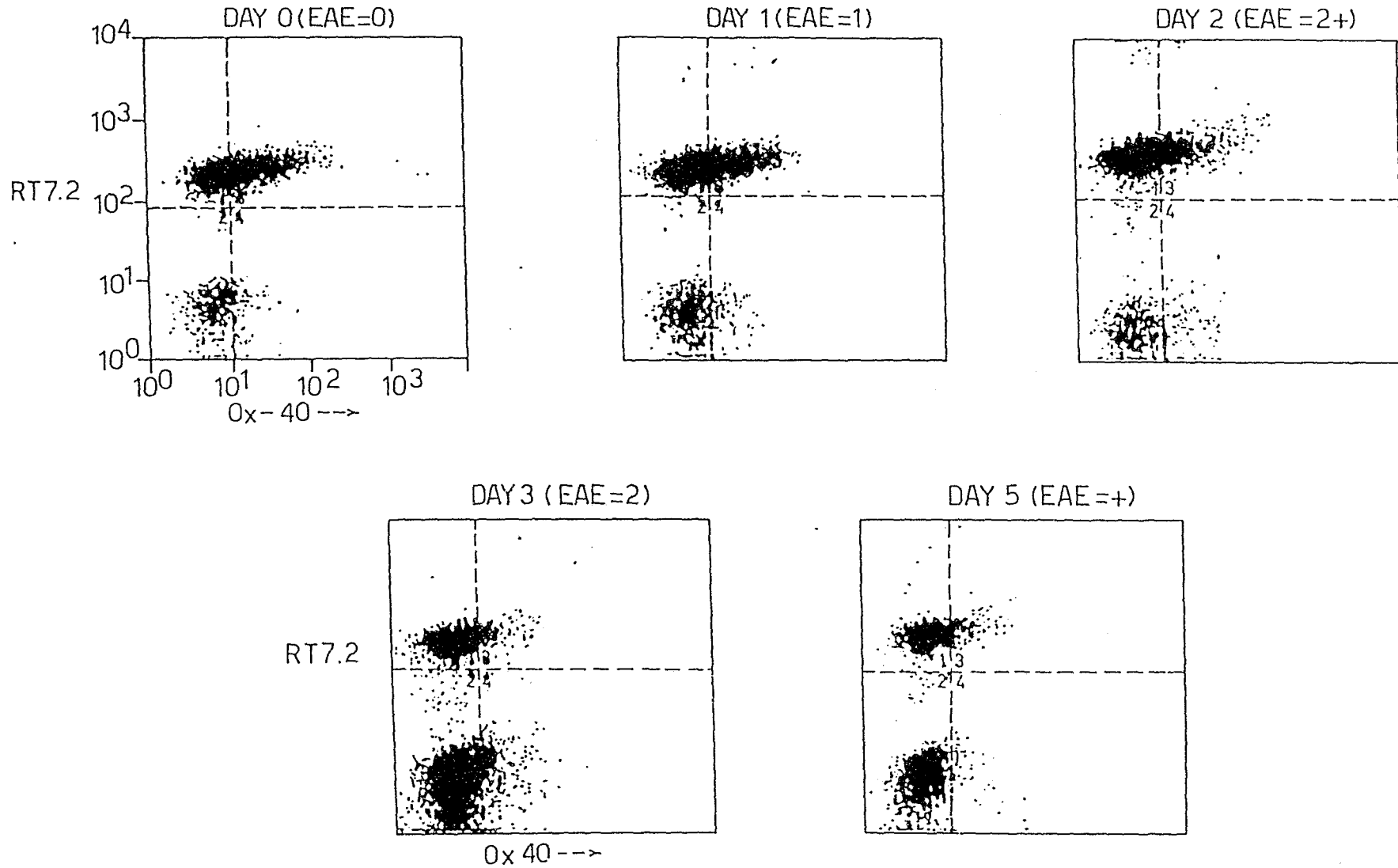
Ox-40 --->

Fig.3.

Vβ PROFILE OF OX-40 PRESORTED,NEGATIVE AND POSITIVE
AT ONSET OF EAE

OX-40-PRESORT SPINAL CORD

Vβ 8.2 RT-PCR =16.7%

OX-40 NEGATIVE SORT SPINAL CORD

Vβ 8.2 RT-PCR =6.1%

OX-40 POSITIVE SORT SPINAL CORD

Vβ 8.2 RT-PCR =54.4%
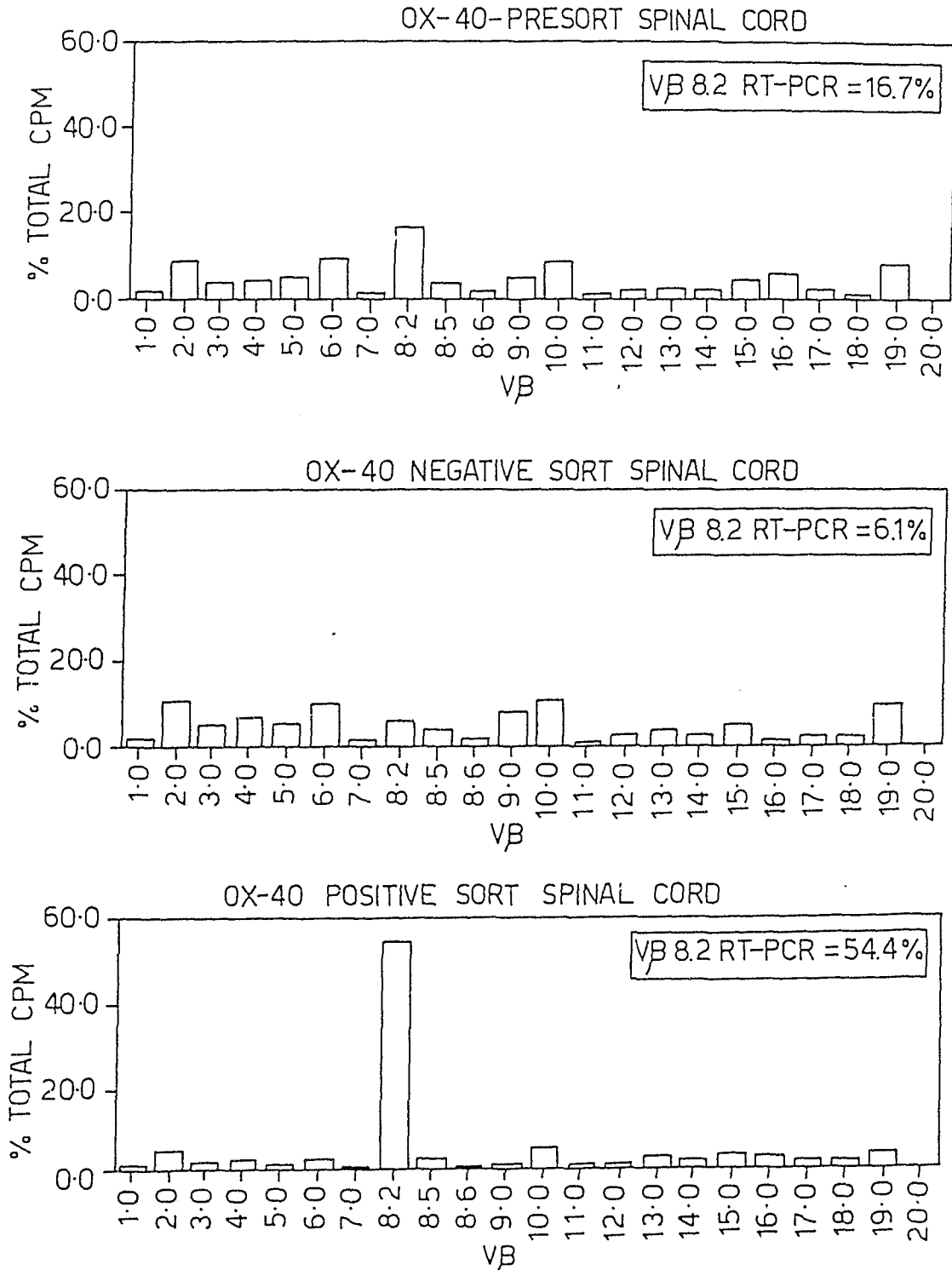
Fig.4.

OX-40 SORT: SPINAL CORD vs LYMPH NODE

Legend:
- ▨ SC OX40+
- ▢ SC OX40−
- ▨ SC NOT SORTED
- ▨ LN OX-40+
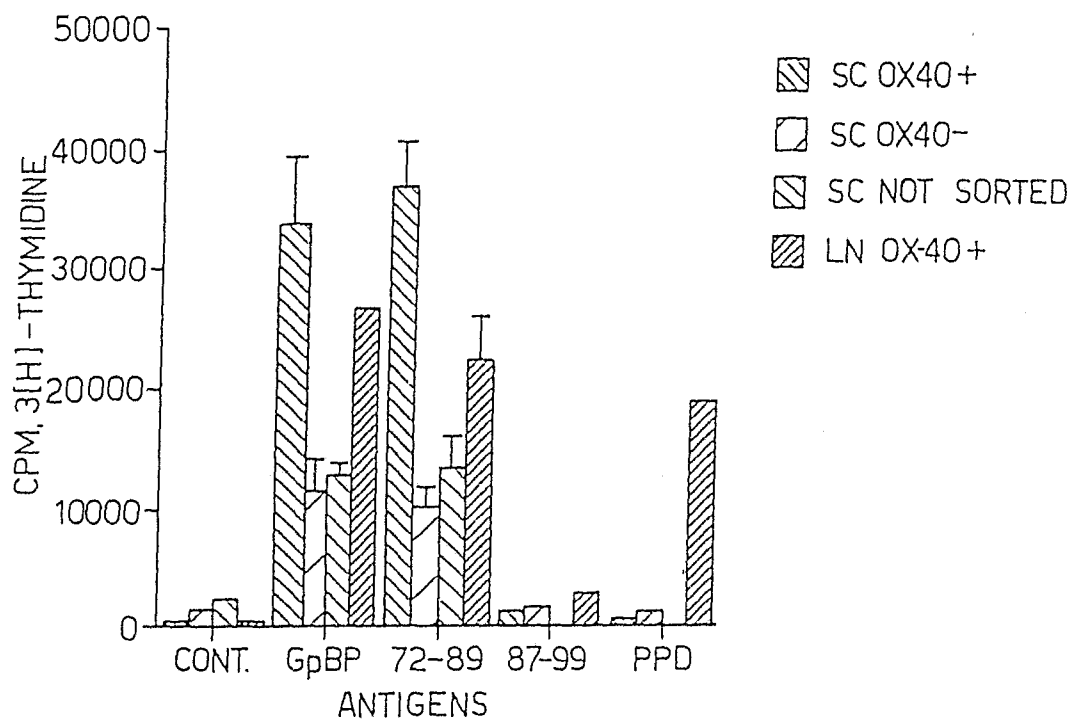
Fig.5.

INHIBITION OF MYELIN BASIC PROTEIN T CELL PROLIFERATION

□ OX-40-RICIN-A

Fig.6A.

EXOTOXIN–OX–40 F1

Fig.6B.

EXOTOXIN OX–40 LEWIS
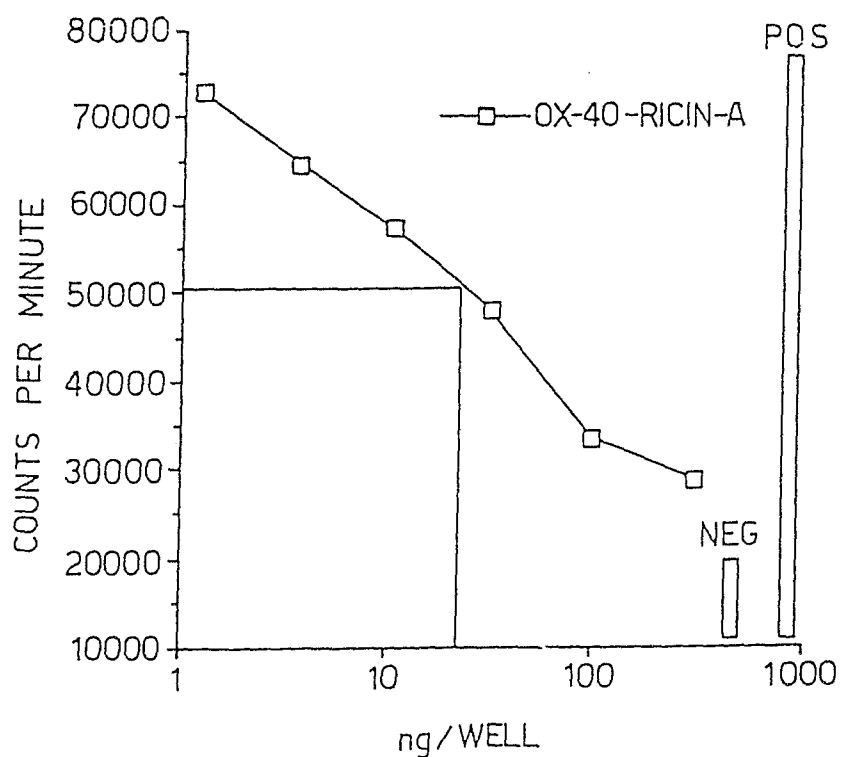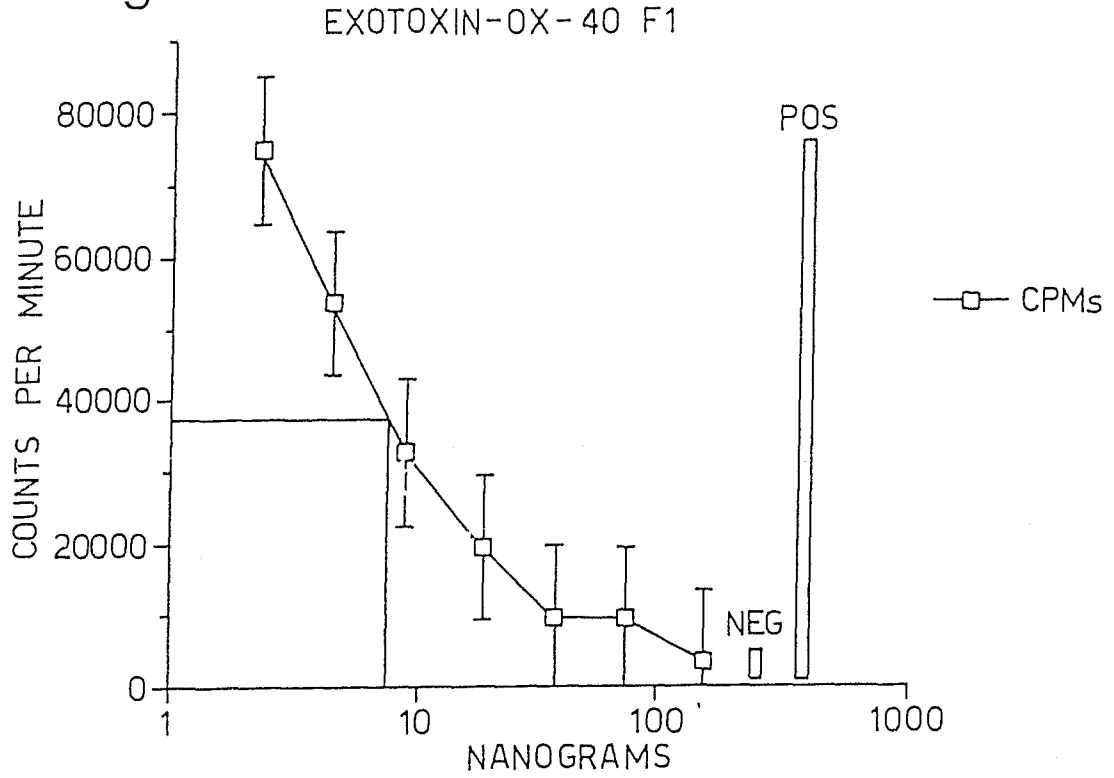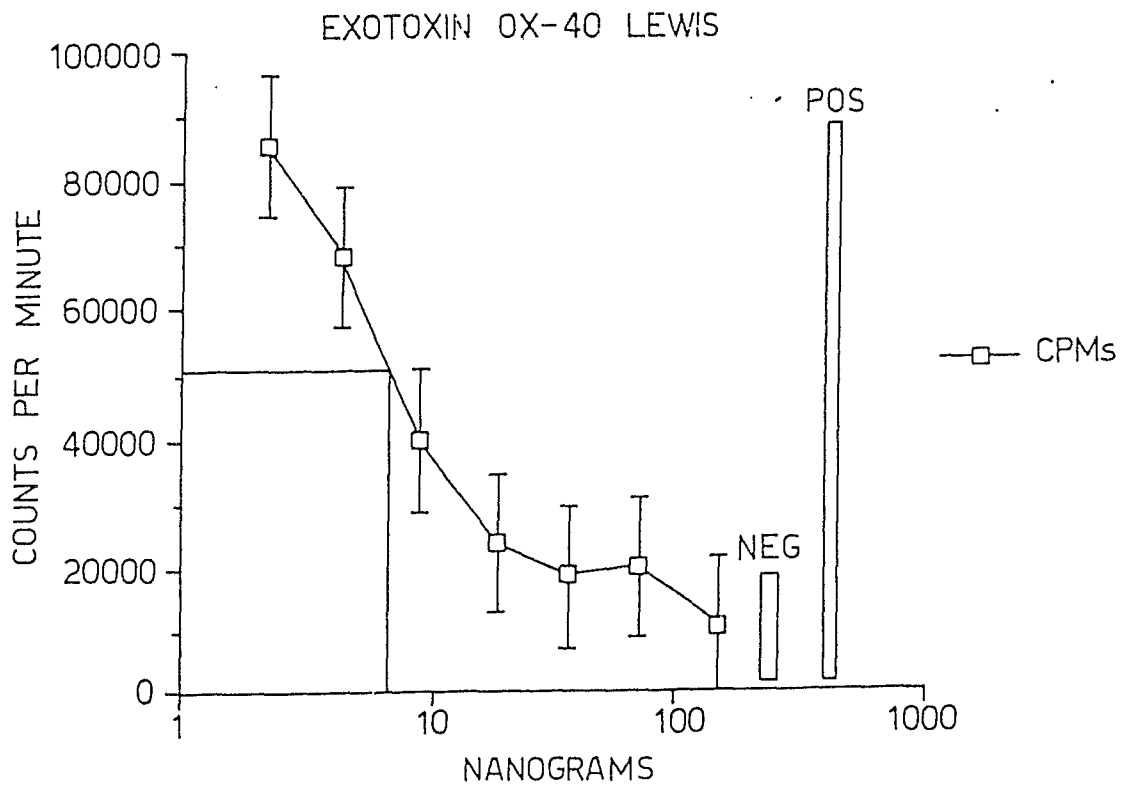
Fig.7. INHIBITION OF PPD T CELL
PROLIFERATION

Fig.9.

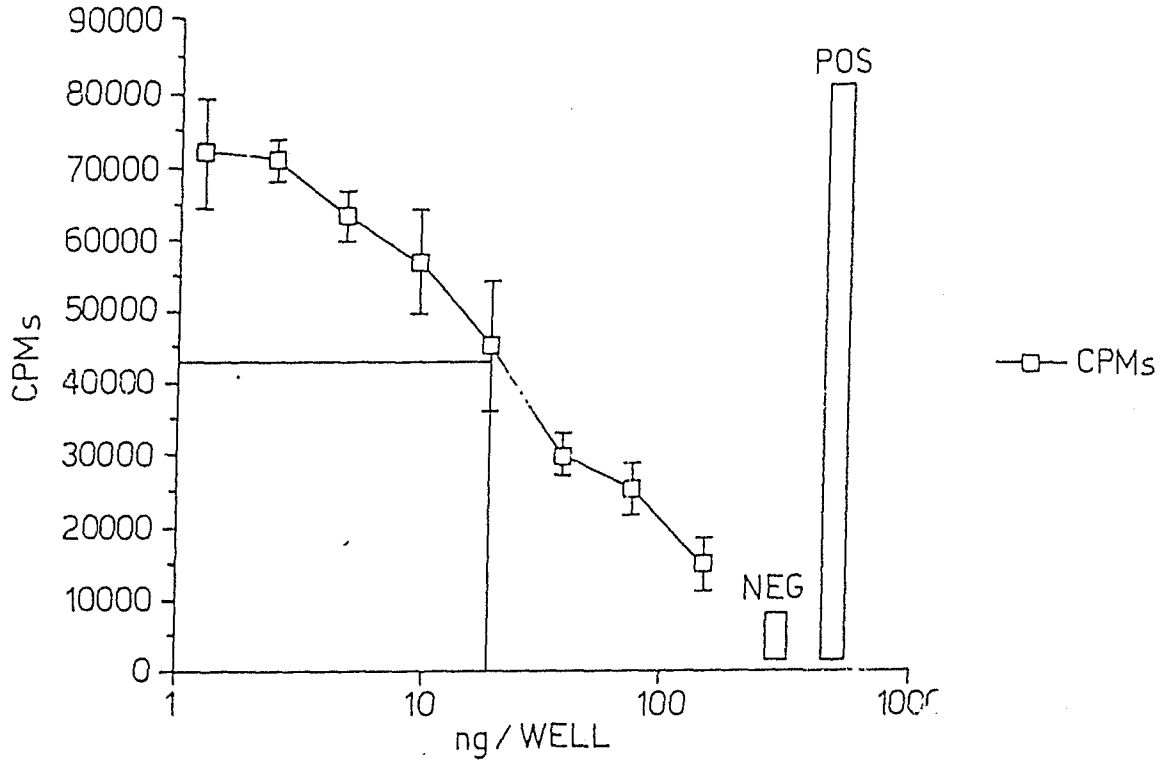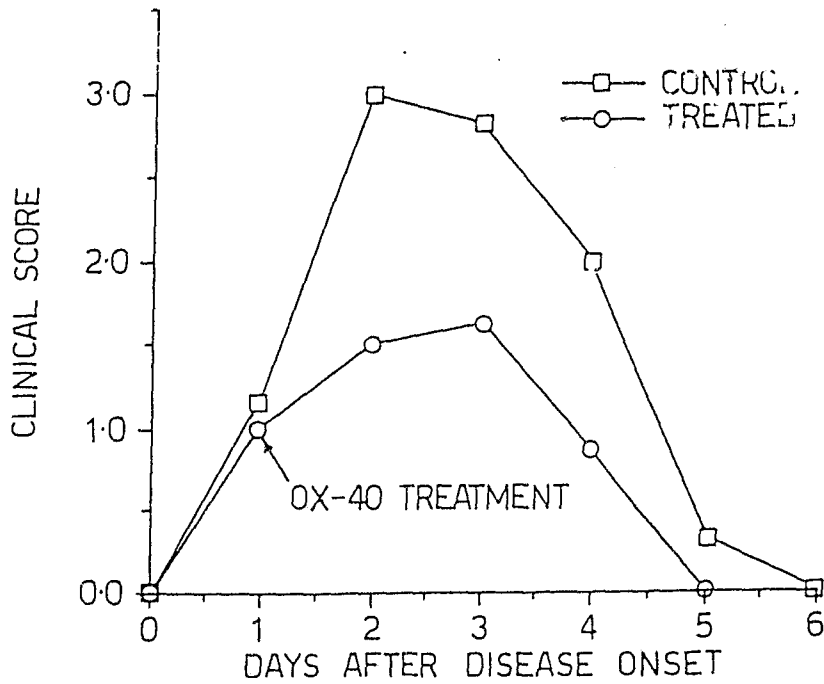OX-40 IMMUNOTOXIN TREATMENT
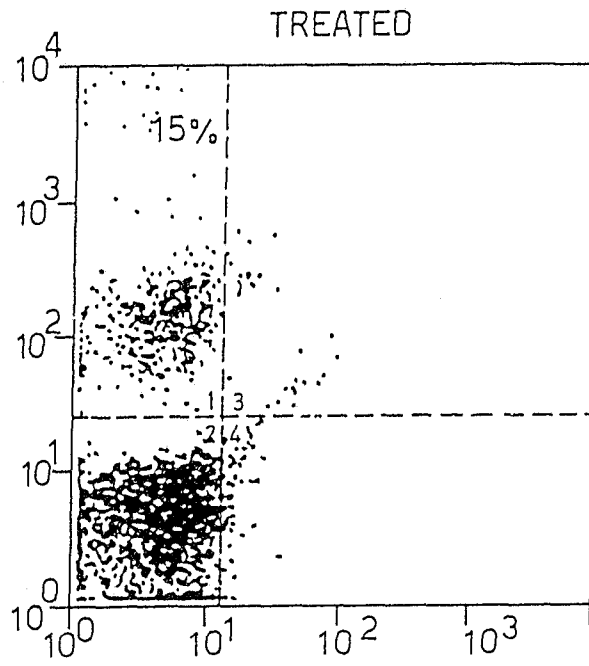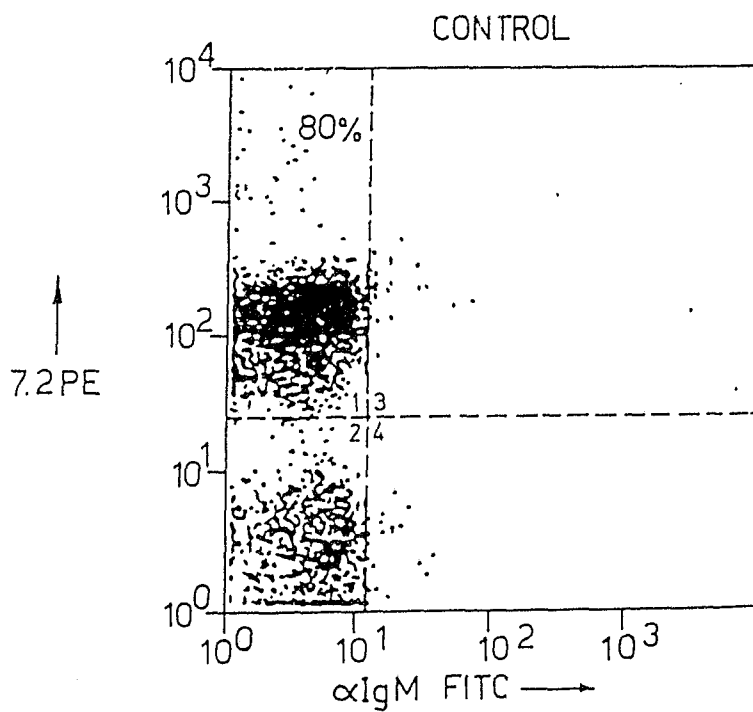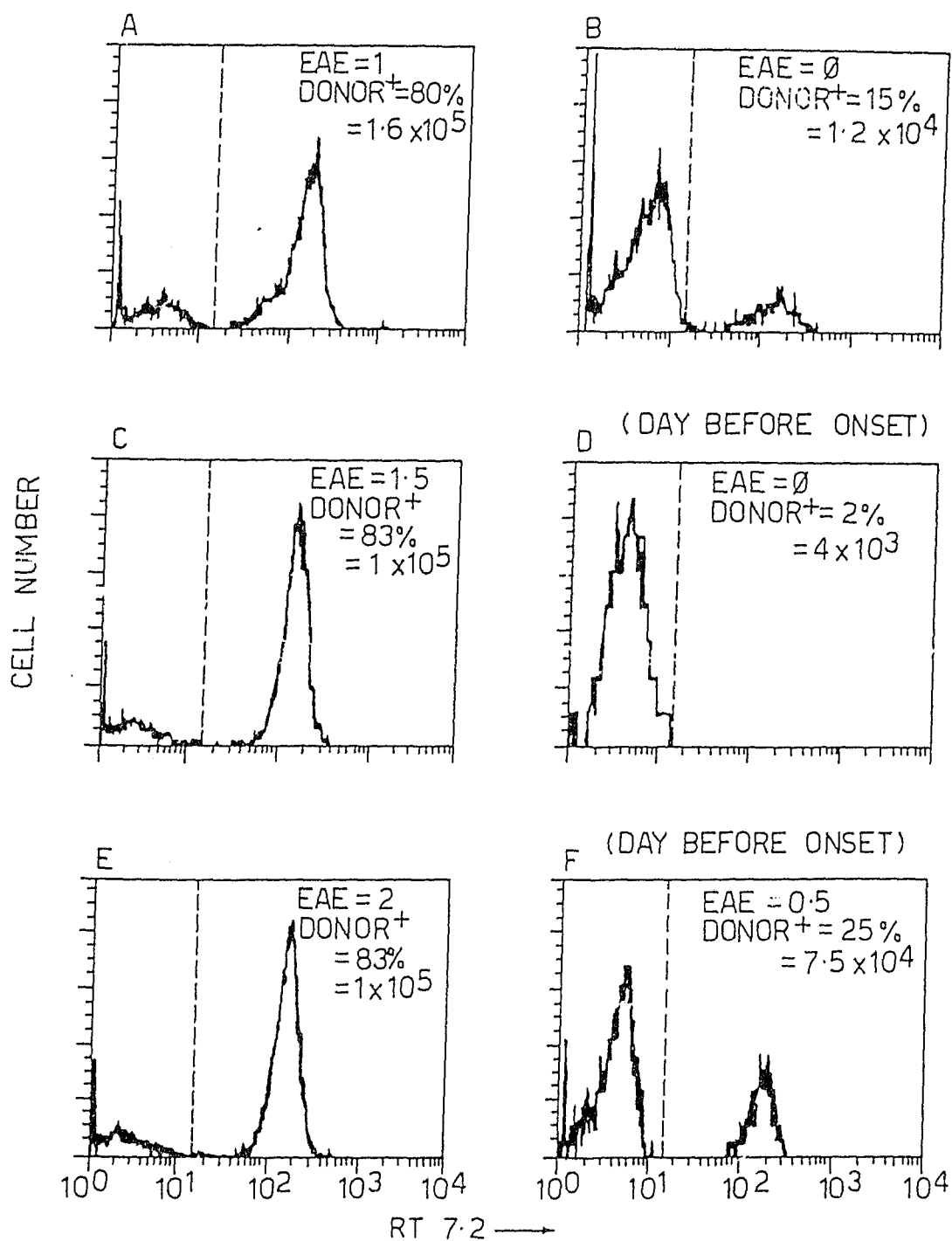
Fig.8A.

TREATED

Fig.8B.

CONTROL

7.2 PE

αIgM FITC ⟶

Fig.10.

OX-40 IMMUNOTOXIN TREATMENT

EFFECT ON DONOR+T-CELL ISOLATED FROM THE SPINAL CORD

CONTROL

TREATED
(SAME DAY AS TRANSFER)

A

EAE =1
DONOR+=80%
=1·6 x10^5

B

EAE = Ø
DONOR+=15%
=1·2 x10^4

C

EAE =1·5
DONOR+
=83%
=1 x10^5

D   (DAY BEFORE ONSET)

EAE =Ø
DONOR+= 2%
=4 x10^3

CELL NUMBER

E

EAE = 2
DONOR+
=83%
=1x10^5

F   (DAY BEFORE ONSET)

EAE =0·5
DONOR+ = 25%
= 7·5 x10^4

10^0 10^1 10^2 10^3 10^4 10^0 10^1 10^2 10^3 10^4

RT 7·2 ⟶

Fig.11.

```
ATG TGC GTG GGG GCT CGG CGG CTG GGC CGC GGG CCG TGT GCG GCT CTG CTC CTC CTG GGC
met cys val gly ala arg arg leu gly arg gly pro cys ala ala leu leu leu leu gly

CTG GGG CTG AGC ACC GTG ACG GGG CTC CAC TGT GTC GGG GAC ACC TAC CCC AGC AAC GAC
leu gly leu ser thr val thr gly leu his cys val gly asp thr tyr pro ser asn asp

CGG TGC TGC CAC GAG TGC AGG CCA GGC AAC GGG ATG GTG AGC CGC TGC AGC CGC TCC CAG
arg cys cys his glu cys arg pro gly asn gly met val ser arg cys ser arg ser gln

AAC ACG GTG TGC CGT CCG TGC GGG CCG GGC TTC TAC AAC GAC GTG GTC AGC TCC AAG CCG
asn thr val cys arg pro cys gly pro gly phe tyr asn asp val val ser ser lys pro

TGC AAG CCC TGC ACG TGG TGT AAC CTC AGA AGT GGG AGT GAA CGG AAG CAG CTA TGC ACG
cys lys pro cys thr trp cys asn leu arg ser gly ser glu arg lys gln leu cys thr

GCC ACA CAG GAC ACA GTC TGT CGC TGC CGG GCG GGC ACC CAG TCC CTG GAC AGC TAC AAG
ala thr gln asp thr val cys arg cys arg ala gly thr gln ser leu asp ser tyr lys

CCT GGA GTT GAC TGT GCC CCC TGC CCT CCA GGG CAC TTC TCC CCA GGC GAC AAC CAG GCC
pro gly val asp cys ala pro cys pro pro gly his phe ser pro gly asp asn gln ala
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TGC AAG CCC TGG ACC ACC TGT ACC TTG GTT GGG AAG CAC ACC CTG CAG CCG GCC AGT AAT
cys lys pro trp thr thr cys thr leu val gly lys his thr leu gln pro ala ser asn

AGC TCG GAC GCA ATC TGT GAG GAC AGG GAC CCC CCA GCC ACG CAG CCC CAG GAG ACC CAG
ser ser asp ala ile cys glu asp arg asp pro pro ala thr gln pro gln glu thr gln

GGT CCC CCG GCC AGG CCC ATC ACT GTC CAG CCC ACT GAA GCC TGG CCC AGA ACC TCA CAG
gly pro pro ala arg pro ile thr val gln pro thr glu ala trp pro arg thr ser gln

GGA CCC TCC ACC CGG TCC GTG GAG GTC CCC GGG GGC CGT GCG GTT GCC GCC ATC CTG GGA
gly pro ser thr arg ser val glu val pro gly gly arg ala val ala ala ile leu gly

CTG GGA CTG GTG CTG GGG CTG CTG GGA CCC CTG GAC ATC CTG CTG GCC CTG TAC CTG ATC
leu gly leu val leu gly leu leu gly pro leu asp ile leu leu ala leu tyr leu ile

CGG AGG GAC CAG AGG CTG CCC CCC GAT GCC CAC AAG CCC CCT GGG GGA GGT AGC TTC CGG
arg arg asp gln arg leu pro pro asp ala his lys pro pro gly gly gly ser phe arg

ACC CCC ATC CAA GAG GAG CAG GCC GAC GCC CAC TCC ACC CTG GTC AAG TTC TGA
thr pro ile gln glu glu gln ala asp ala his ser thr leu val lys phe OPA
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<p style="text-align:right">10/10</p>

Fig.11. (Cont.)