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(54) **SOLUBLE POLYPEPTIDE FRACTIONS OF THE LAG-3 PROTEIN, PRODUCTION METHOD, THERAPEUTIC COMPOSITION, ANTI-IDIOTYPE ANTIBODIES**

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PCT Pub. Date: **Nov. 16, 1995**

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Appl. No.: **08/737,271**
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(52) **U.S. Cl.** **435/69.1**; 435/455; 530/324; 530/330; 530/350; 530/387.3; 530/391.3; 530/391.7; 530/861

(58) **Field of Search** 435/69.1, 455; 530/324, 330, 350, 387.3, 391.3, 391.7, 861

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Primary Examiner—David Saunders

(74) *Attorney, Agent, or Firm*—Browdy and Neimark, PLLC

(57) **ABSTRACT**

Soluble polypeptide fraction consisting of all or part [one] *of* at least *one* of the four immunoglobulin-type extracellular LAG-3 protein domains (amino acids 1–159, 160–[230] *239*, 240–330 and 331–412 of the SEQ ID NO:1 sequence) or consisting of one peptide sequence derived from these domains by replacement, addition or deletion of one or more amino acids. The fraction of the invention has a specificity at least equal to that of LAG-3 in relation to its ligand.

8 Claims, 12 Drawing Sheets

FIG. 1

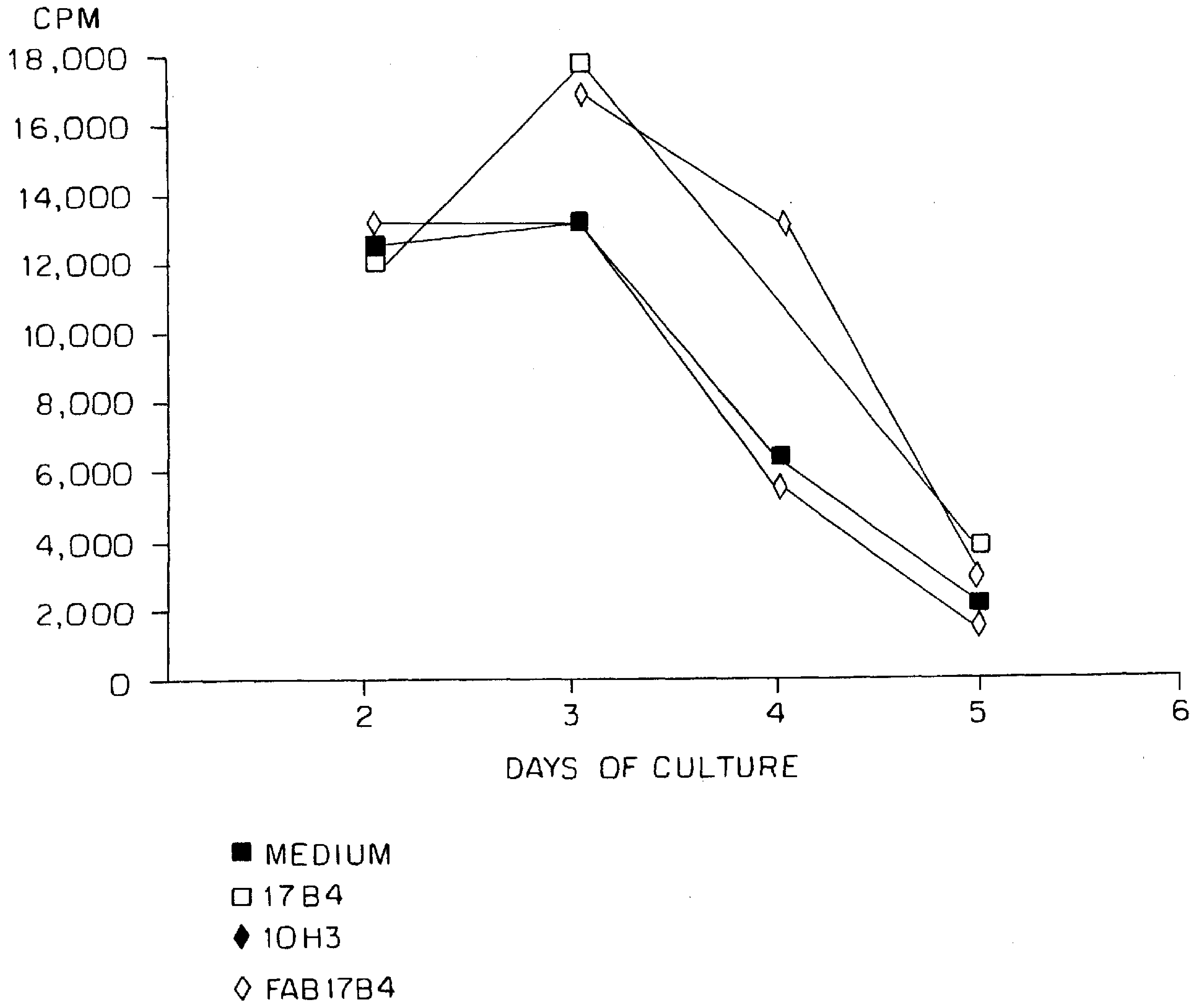

CPM
18,000
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
2
3
4
5
6
DAYS OF CULTURE
■ MEDIUM
□ 17B4
♦ 10H3
◇ FAB17B4

FIG. 2

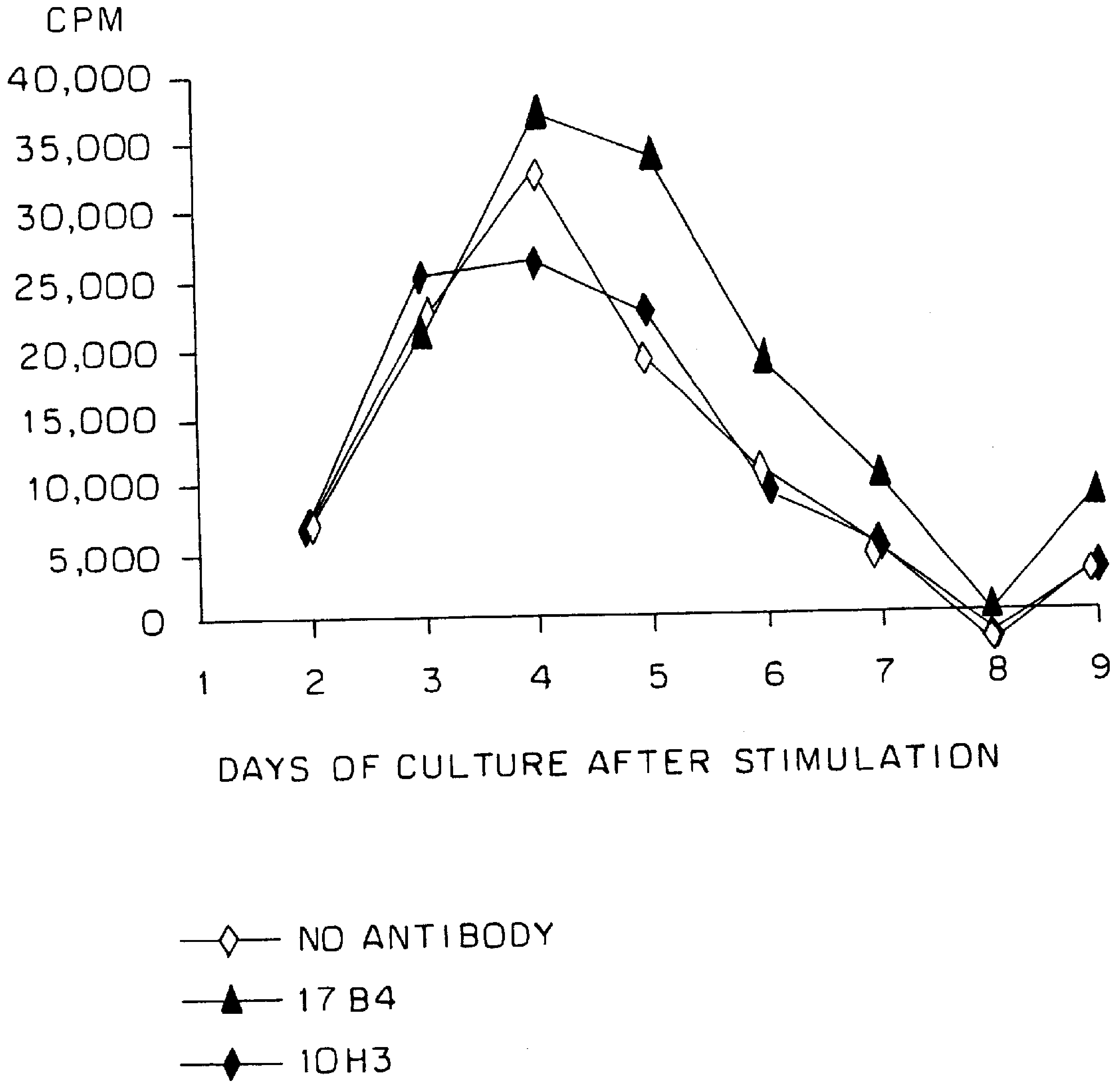

CPM
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
1
2
3
4
5
6
7
8
9
DAYS OF CULTURE AFTER STIMULATION
◇ NO ANTIBODY
▲ 17 B4
◆ 10H3

FIG. 3

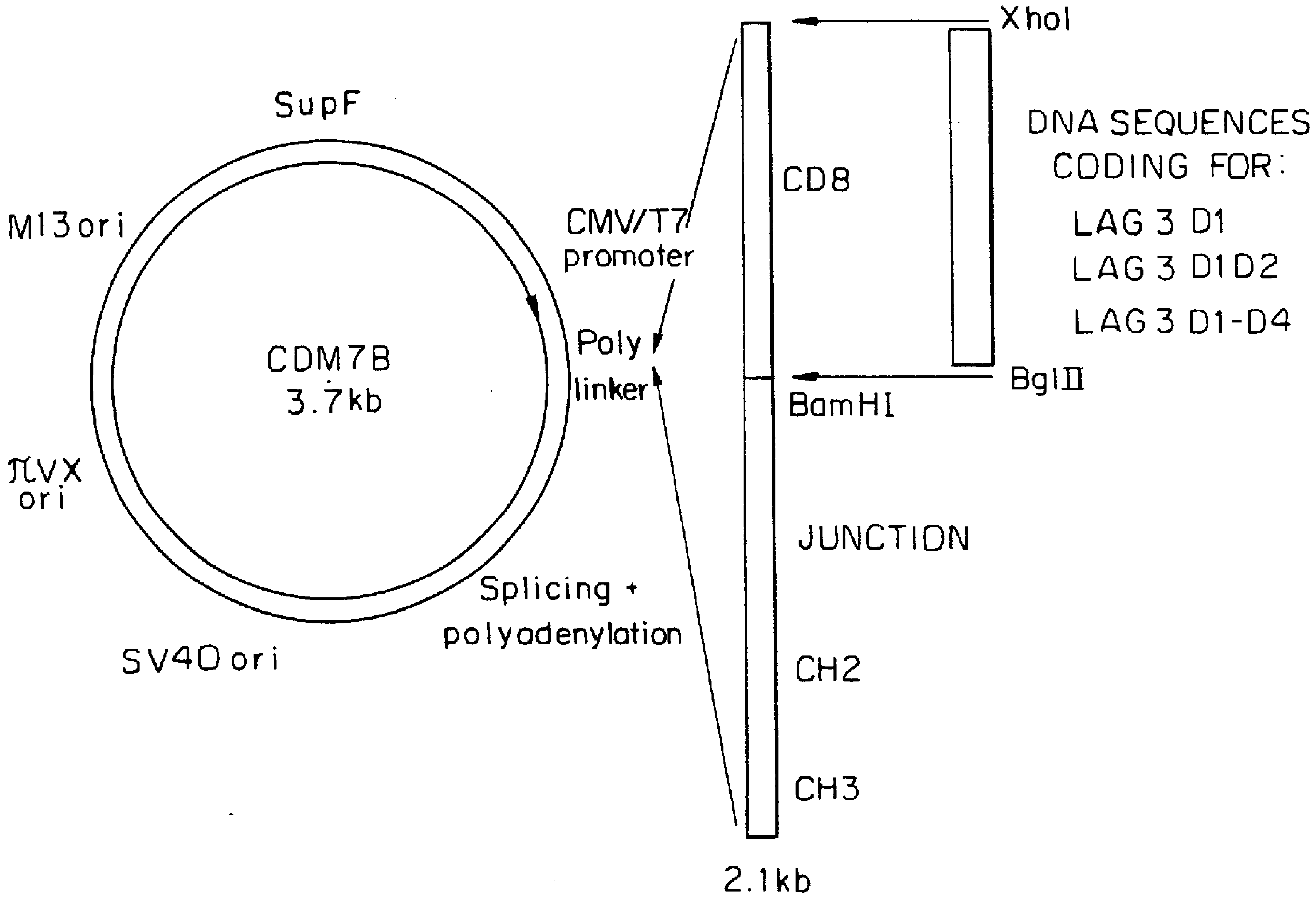

SupF
M13ori
CDM7B
3.7kb
πVX
ori
SV40ori
Splicing +
polyadenylation
CMV/T7
promoter
Poly
linker
CD8
BamHI
JUNCTION
CH2
CH3
2.1kb
XhoI
DNA SEQUENCES
CODING FOR:
LAG 3 D1
LAG 3 D1D2
LAG 3 D1-D4
BglII

FIG. 4

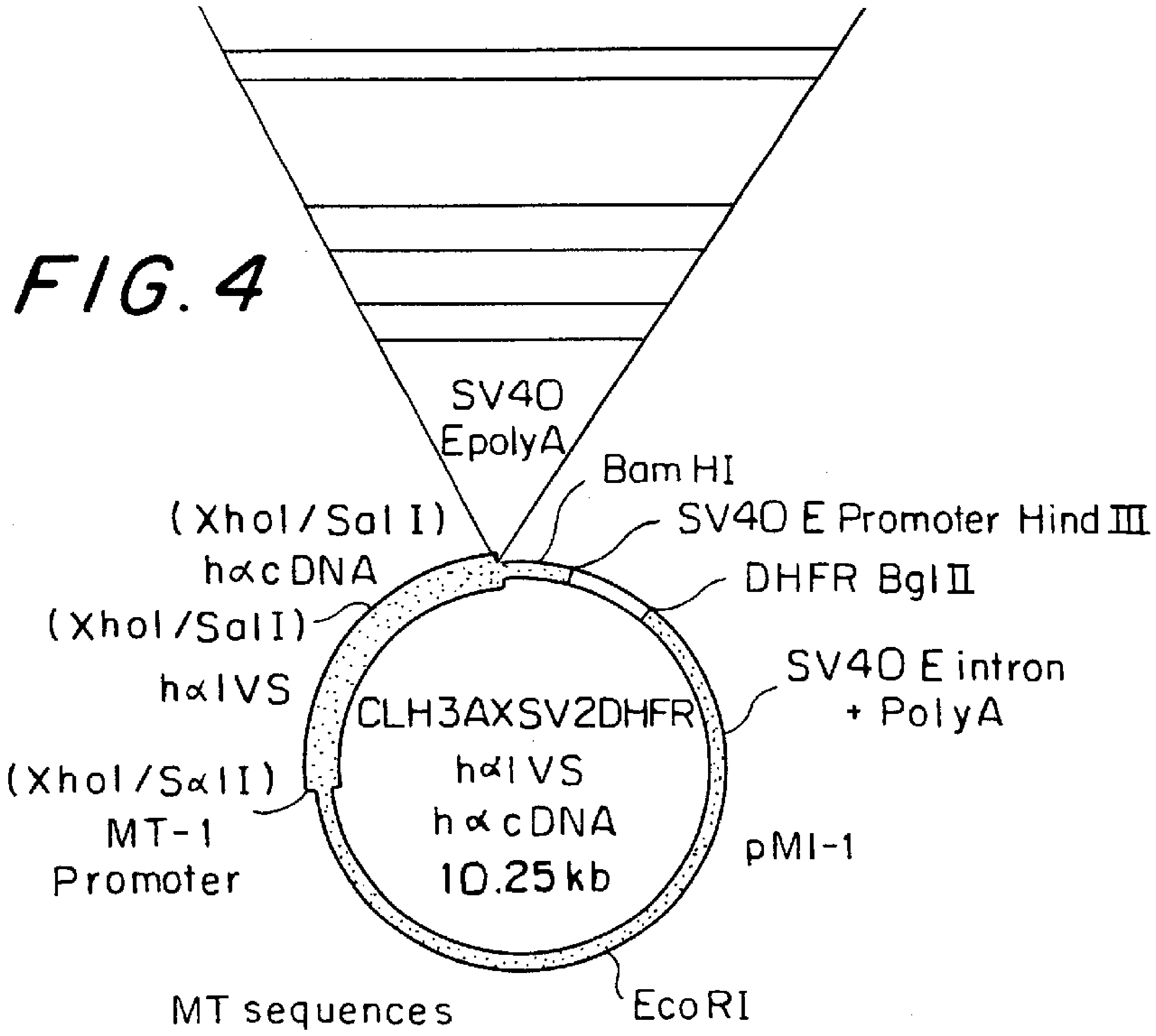

SV40
EpolyA
Bam HI
SV40 E Promoter Hind III
DHFR BglII
SV40 E intron
+ PolyA
pMI-1
EcoRI
MT sequences
MT-1
Promoter
(XhoI/SαII)
hαIVS
(XhoI/SalI)
hαcDNA
(XhoI/Sal I)
CLH3AXSV2DHFR
hαIVS
hαcDNA
10.25kb

FIG. 5

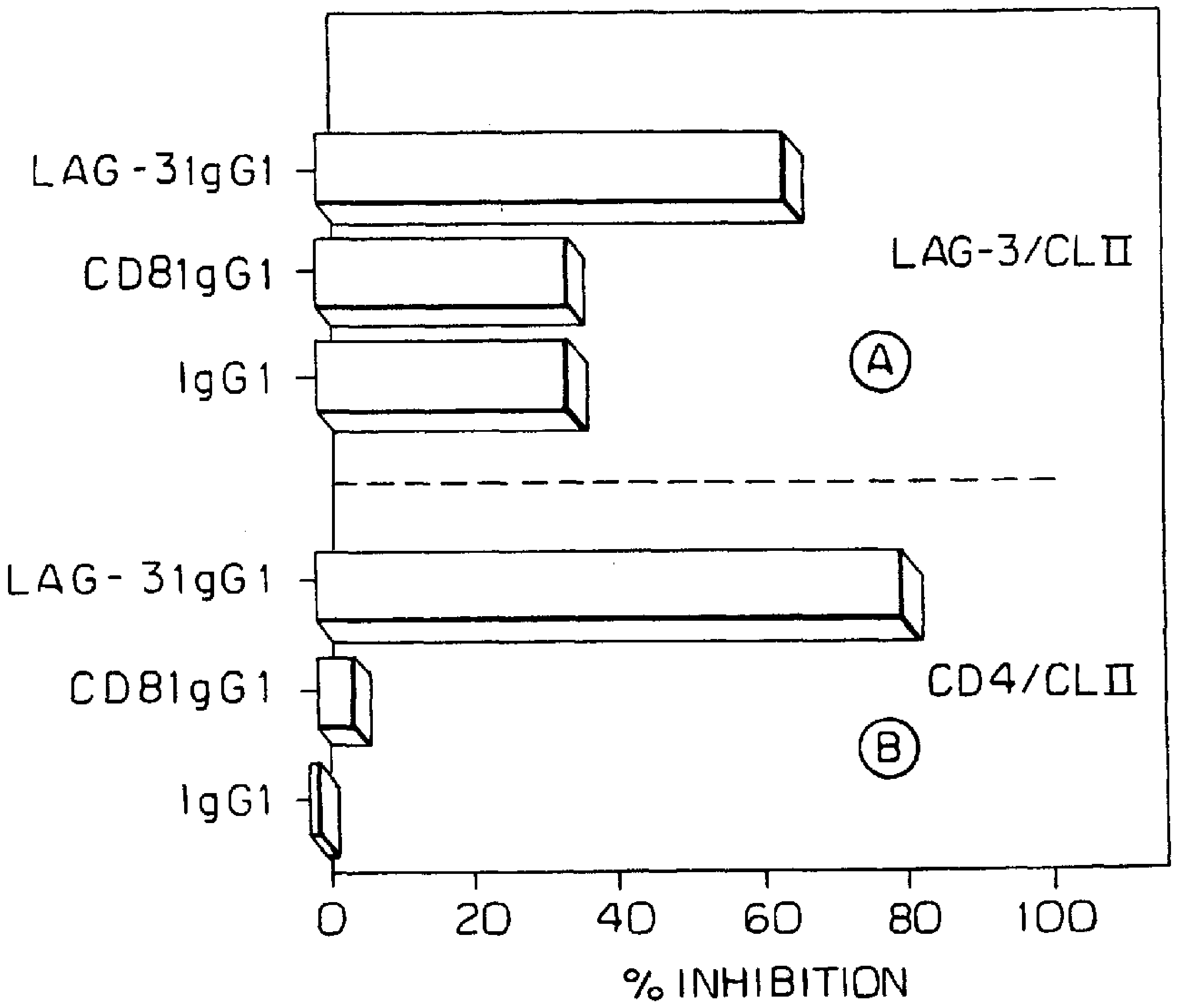

LAG-3IgG1
CD8IgG1
IgG1
LAG-3/CLII
(A)
LAG-3IgG1
CD8IgG1
IgG1
CD4/CLII
(B)
0
20
40
60
80
100
% INHIBITION

FIG. 6

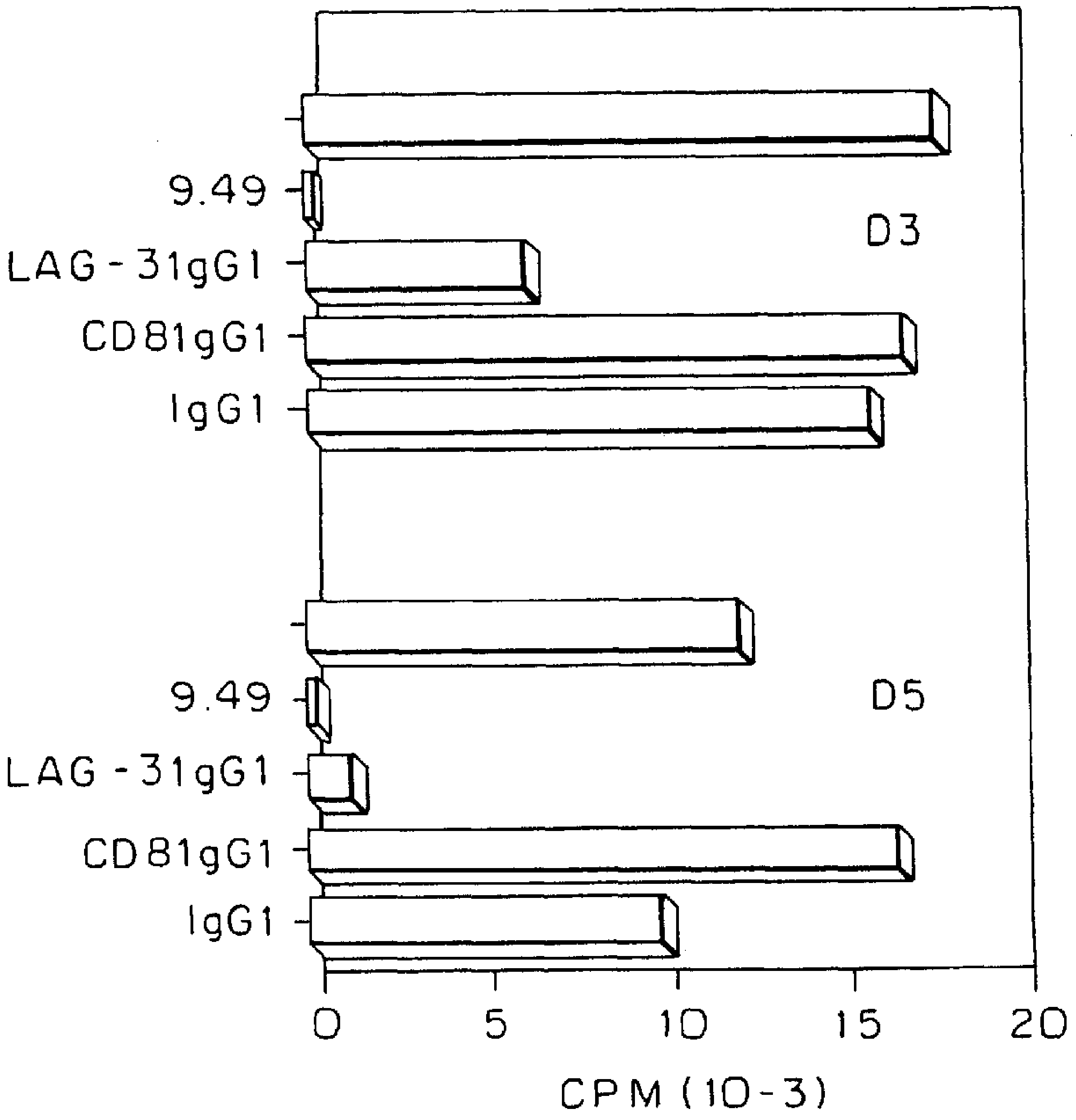
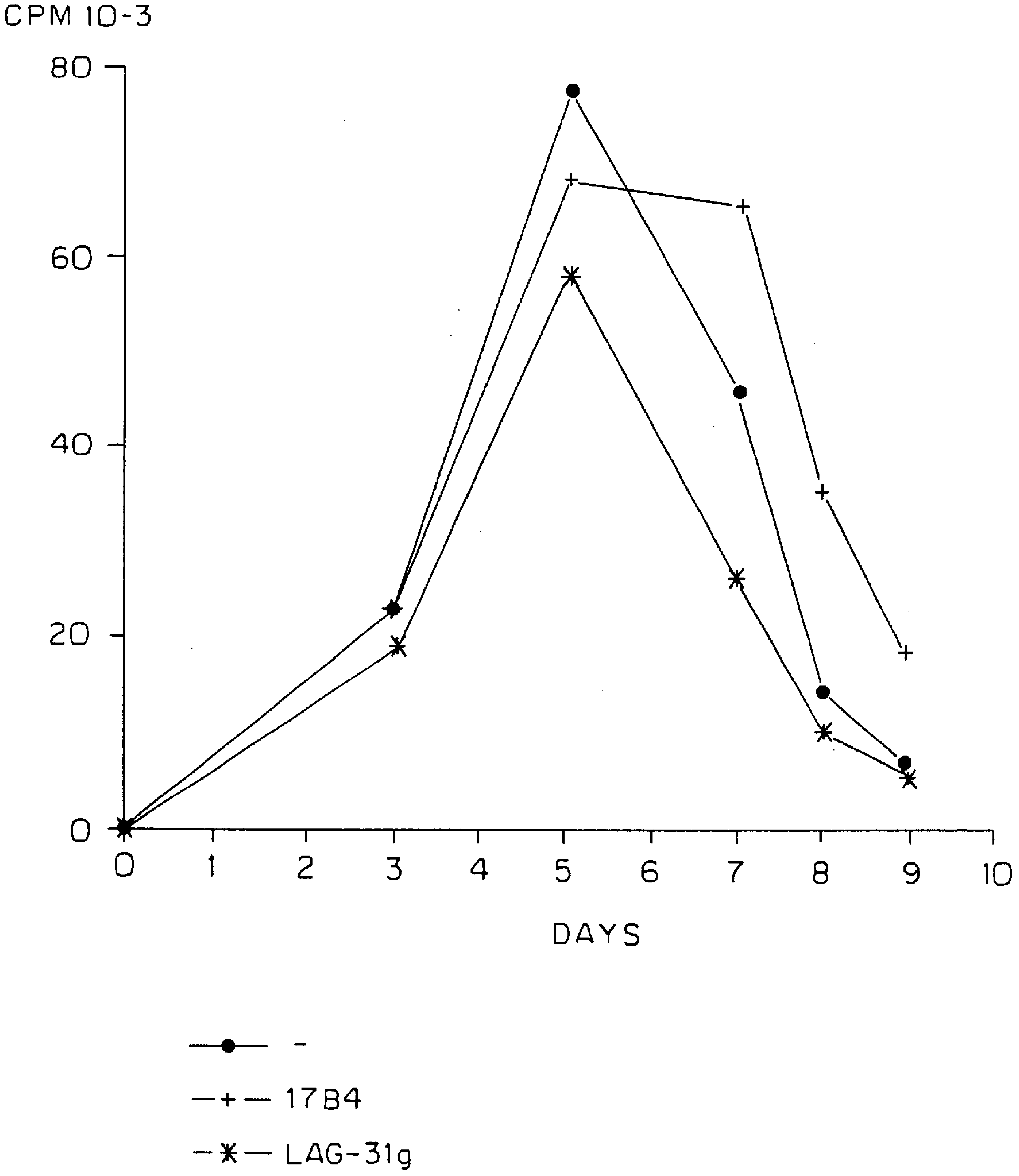

9.49
LAG-3IgG1
CD8IgG1
IgG1
D3
9.49
LAG-3IgG1
CD8IgG1
IgG1
D5
0
5
10
15
20
CPM (10-3)

FIG.7



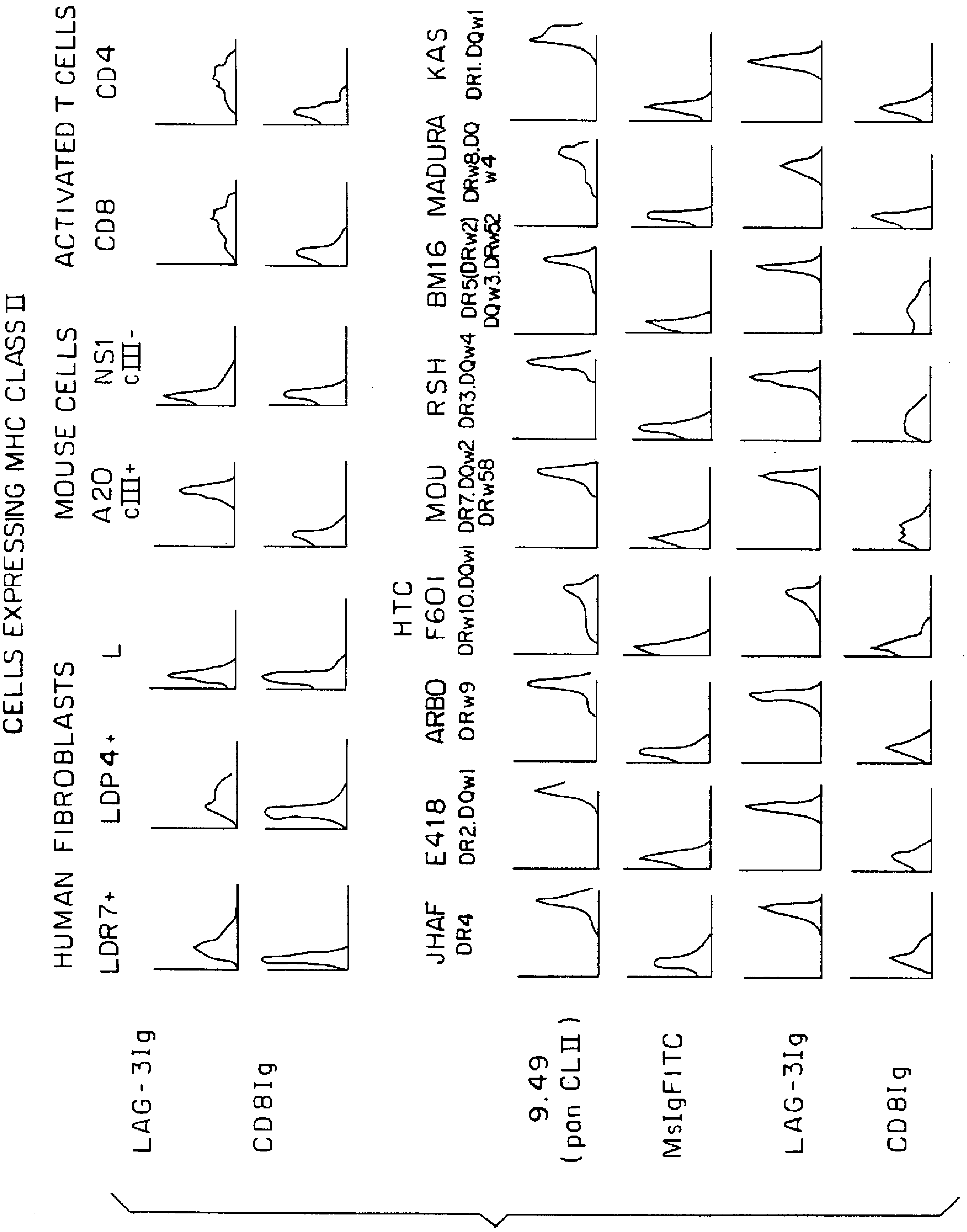

CELLS EXPRESSING MHC CLASS II
HUMAN FIBROBLASTS
LDR7+
LDP4+
L
MOUSE CELLS
A20
cIII+
NS1
cIII-
ACTIVATED T CELLS
CD8
CD4
LAG-3Ig
CD8Ig
HTC
JHAF
DR4
E418
DR2.DQw1
ARBO
DRw9
F6O1
DRw10.DQw1
MOU
DR7.DQw2
DRw58
RSH
DR3.DQw4
BM16
DR5(DRw2)
DQw3.DRw52
MADURA
DRw8.DQ
w4
KAS
DR1.DQw1
9.49
(pan CL II)
MsIgFITC
LAG-3Ig
CD8Ig

FIG. 8

FIG. 9

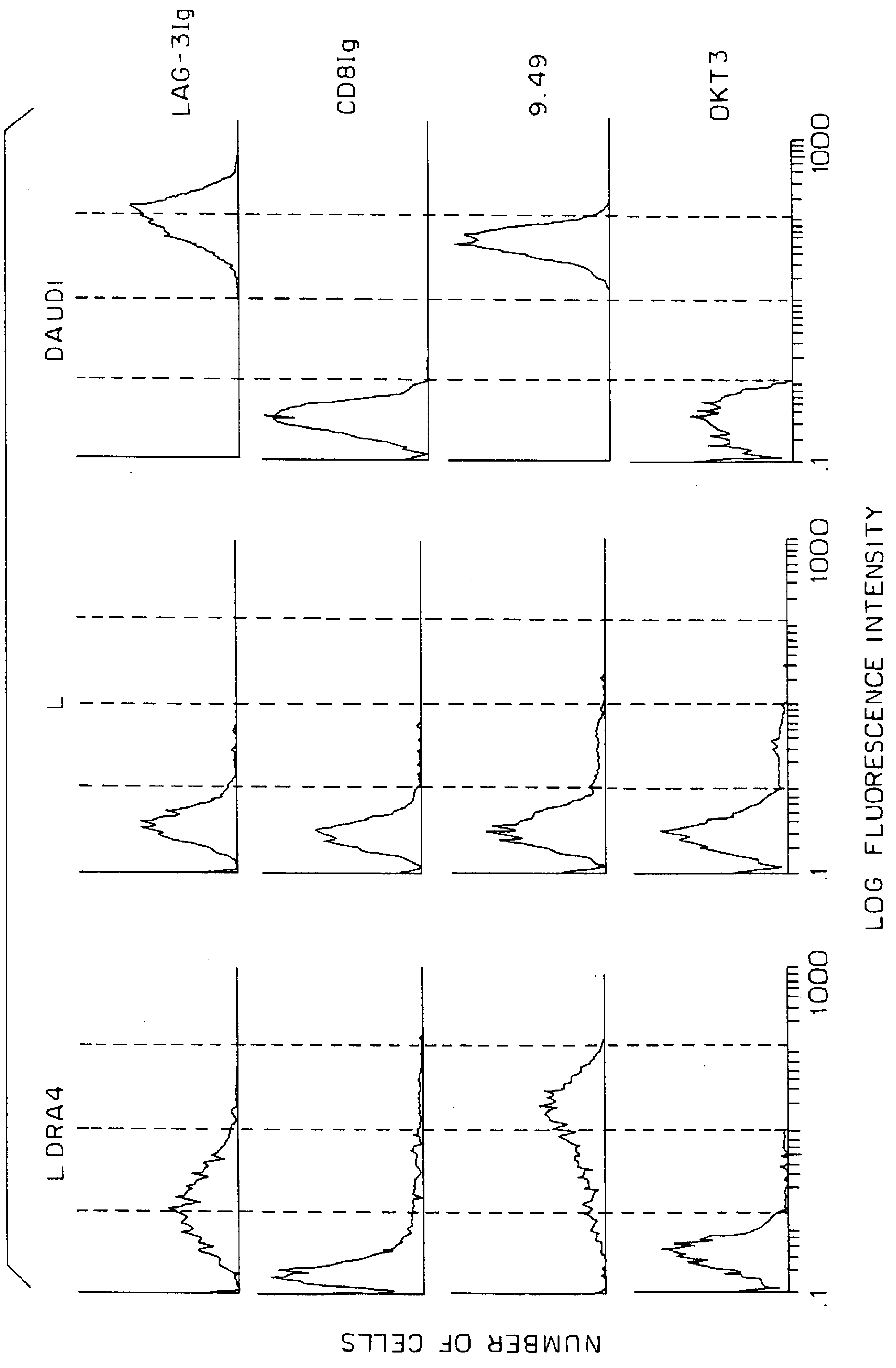

LDRA4
L
DAUDI
LAG-3Ig
CD8Ig
9.49
OKT3
.1
1000
.1
1000
.1
1000
LOG FLUORESCENCE INTENSITY
NUMBER OF CELLS

FIG. 10

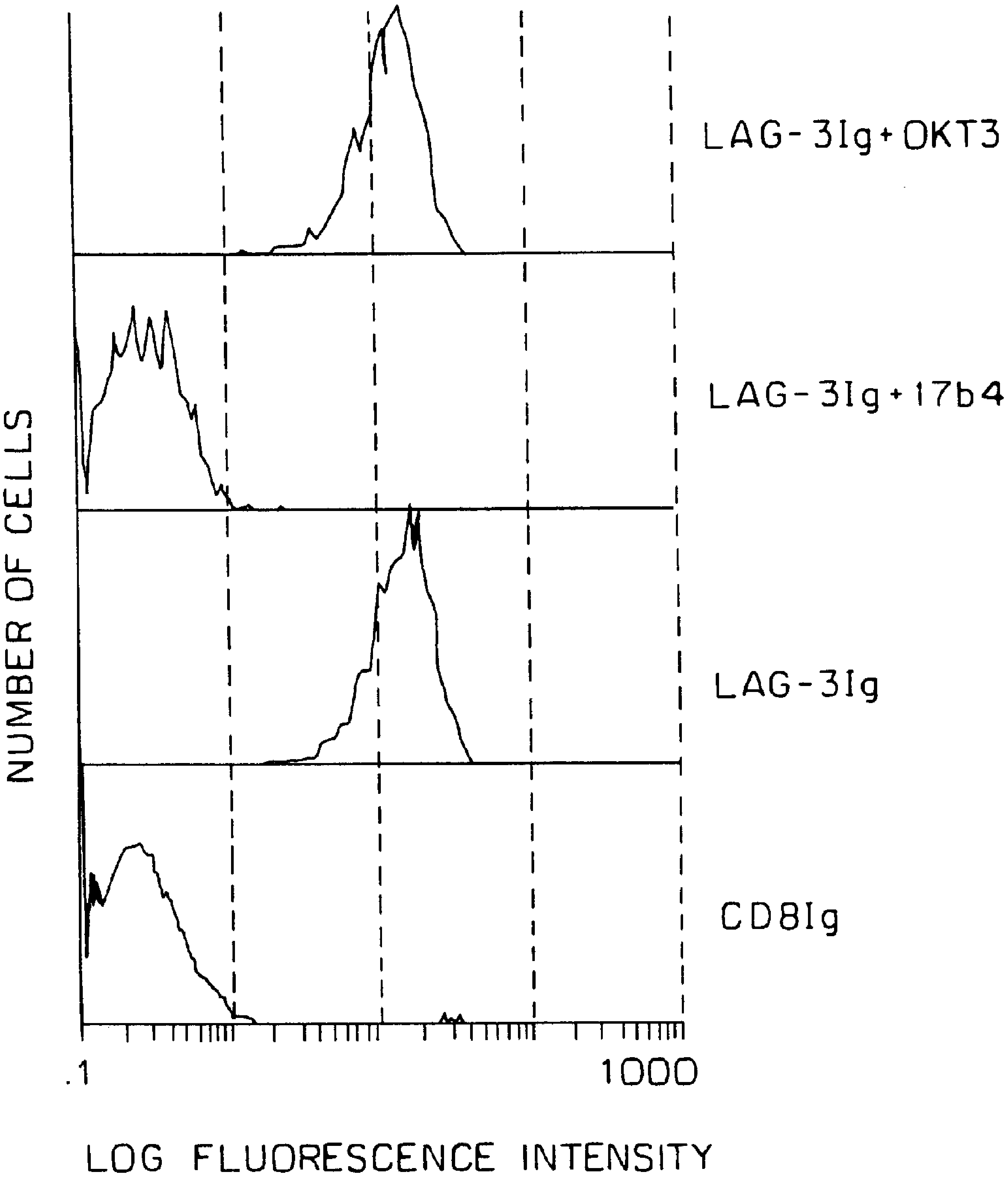

NUMBER OF CELLS
LAG-3Ig+OKT3
LAG-3Ig+17b4
LAG-3Ig
CD8Ig
.1
1000
LOG FLUORESCENCE INTENSITY

FIG. 11A

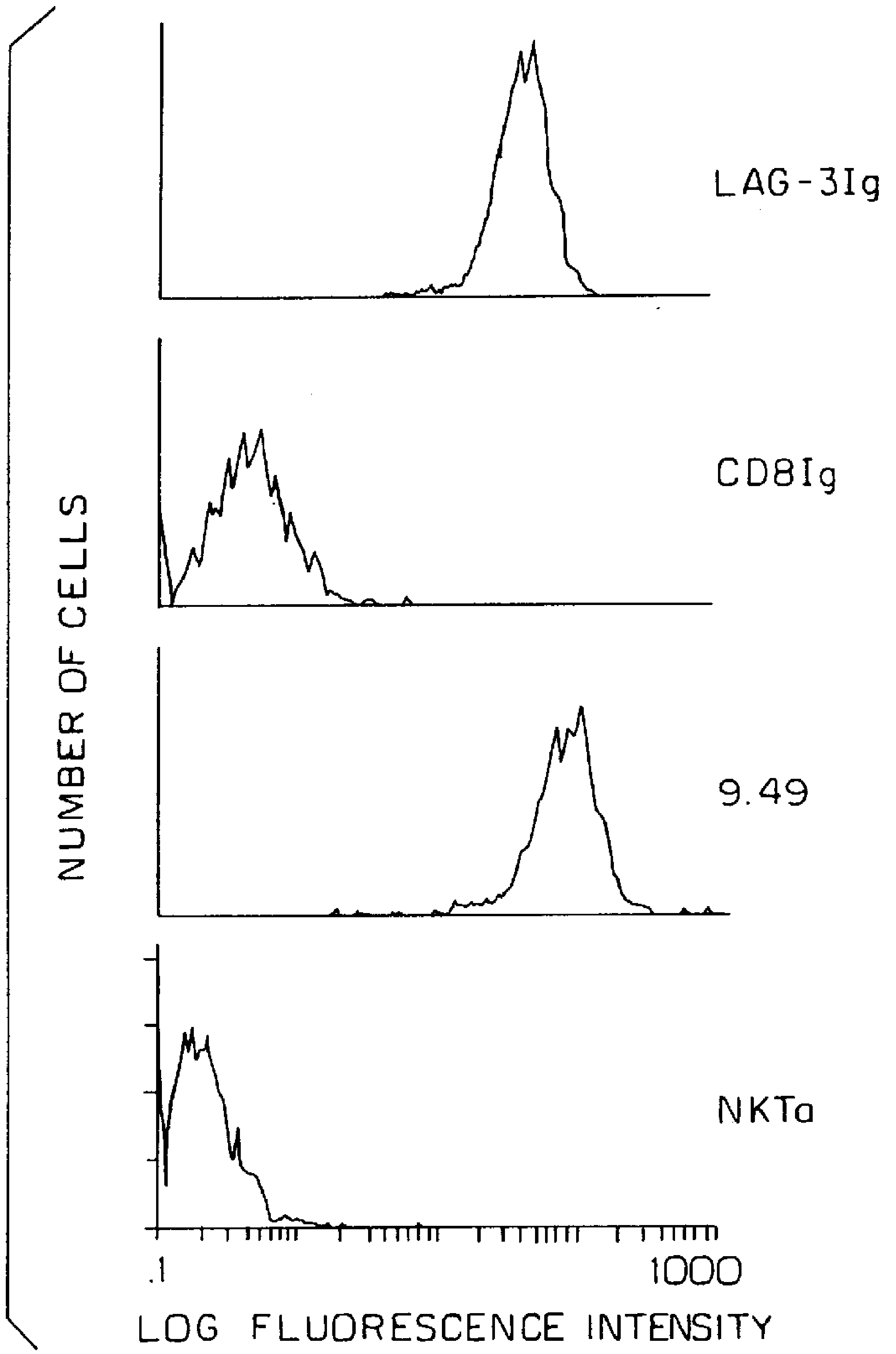


FIG. 11B

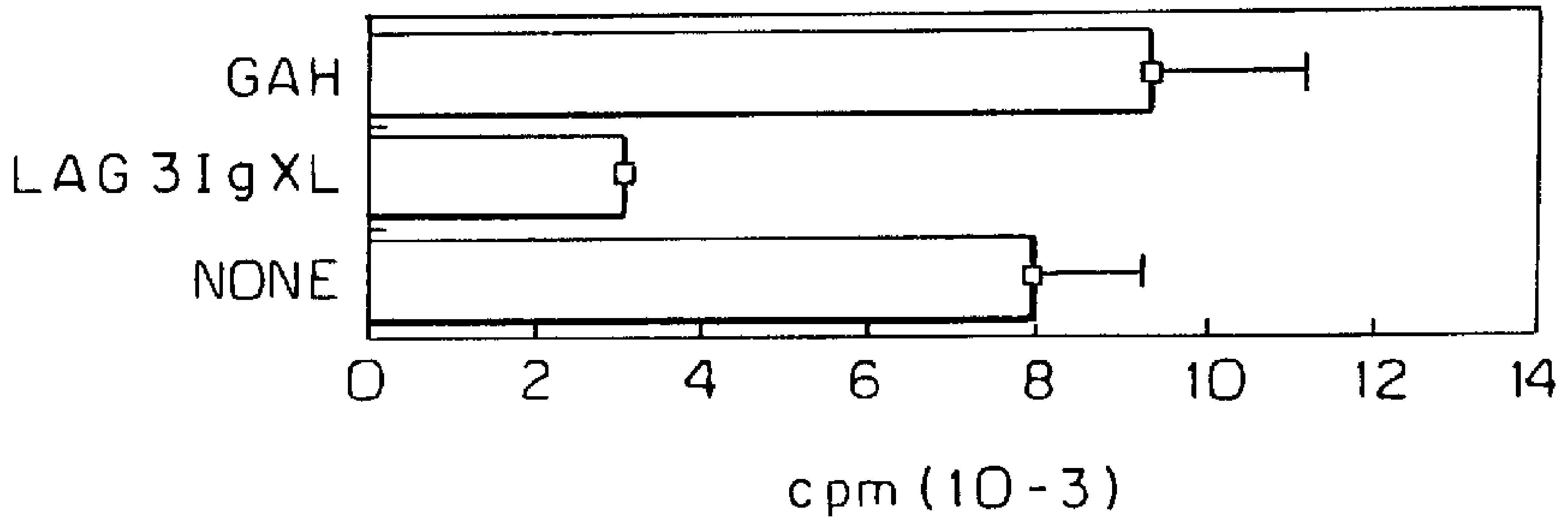


FIG. 11C

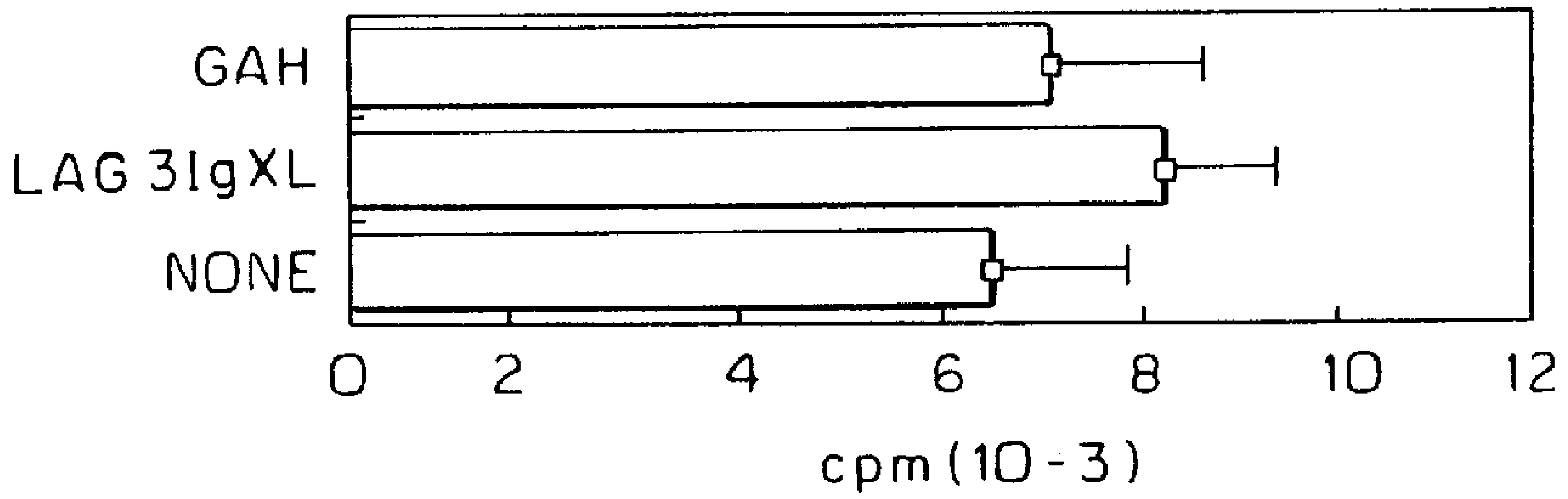


FIG. 12

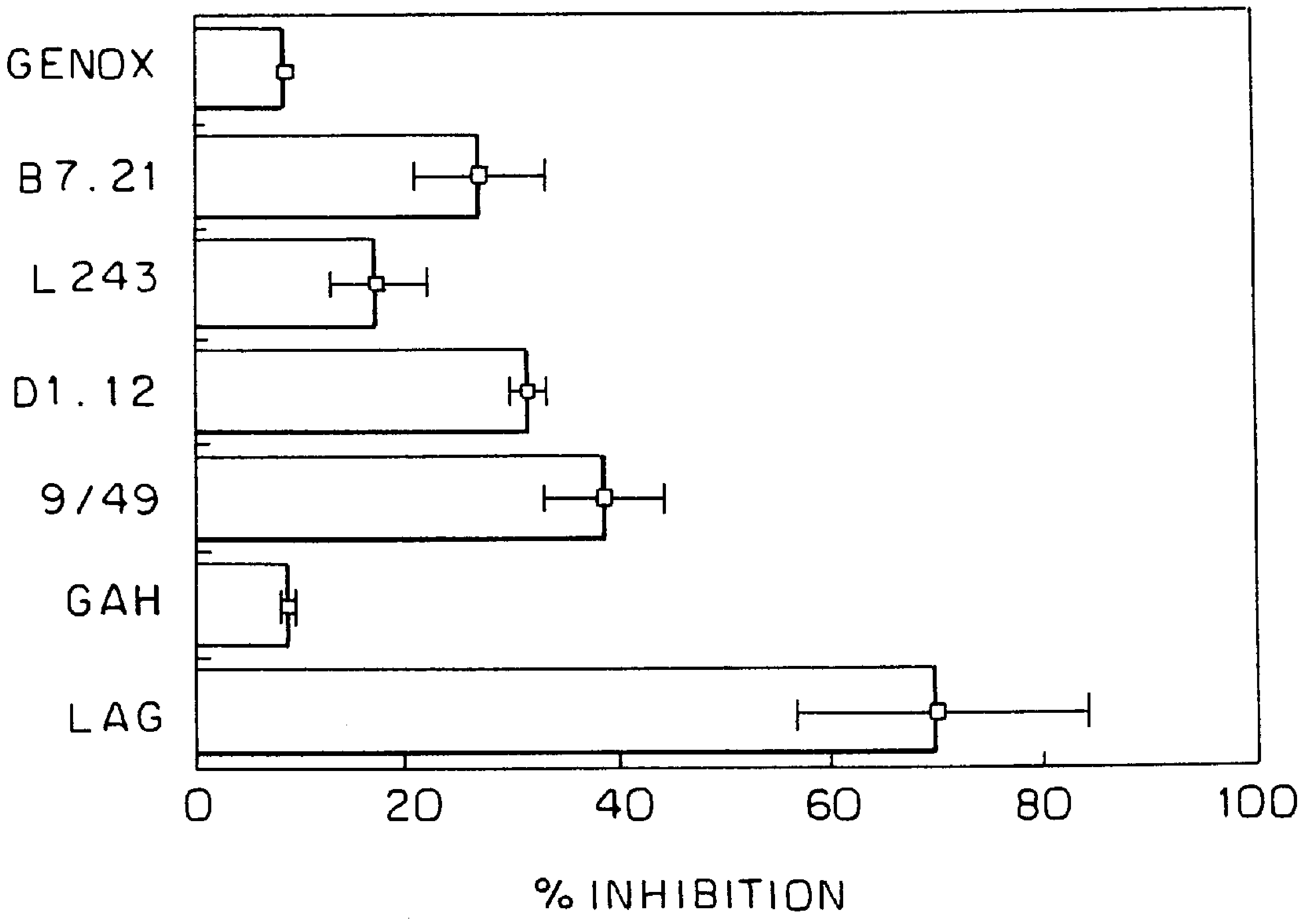


FIG. 13A

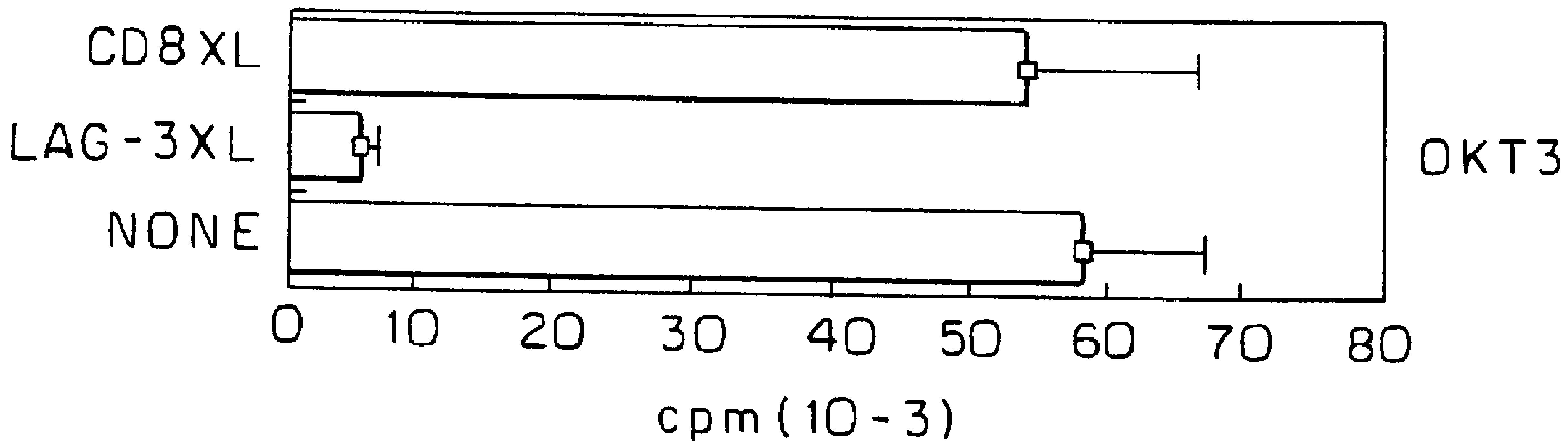


FIG. 13B

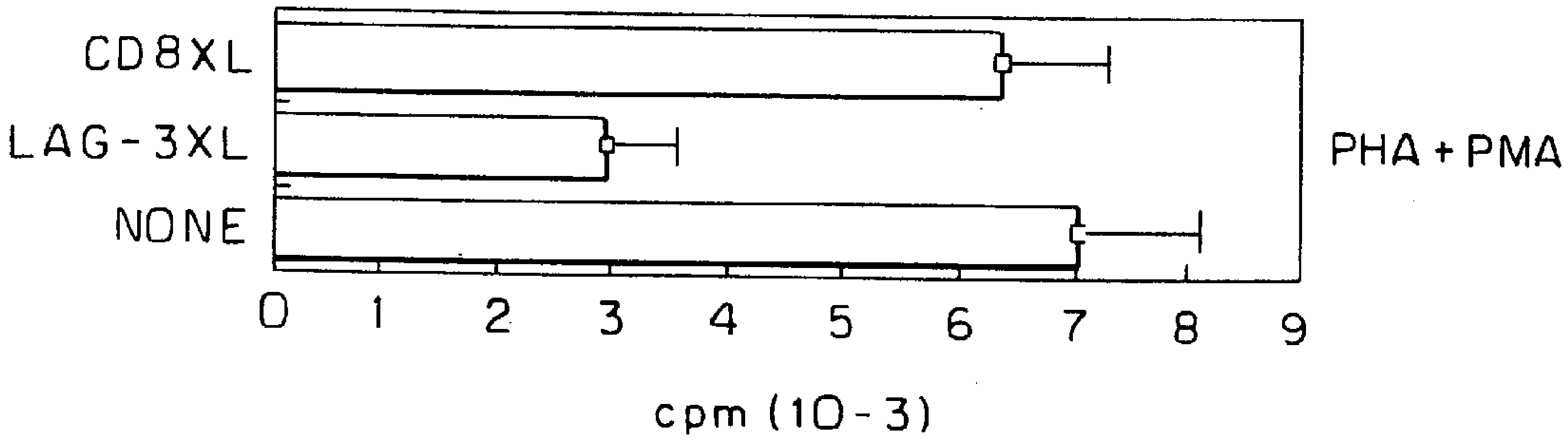


FIG. 13C

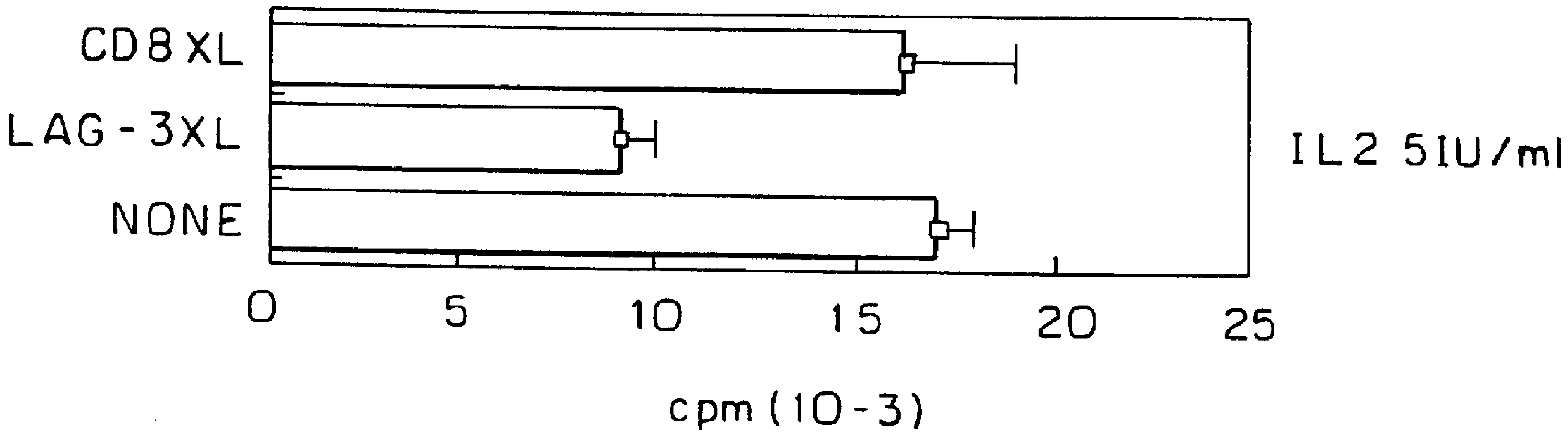


FIG. 13D

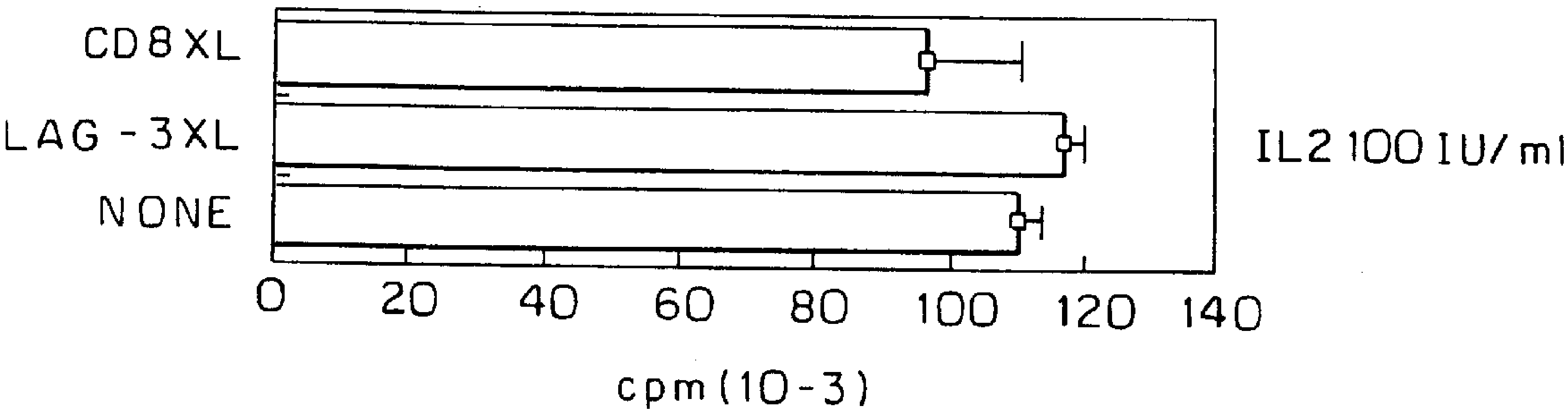


FIG. 14

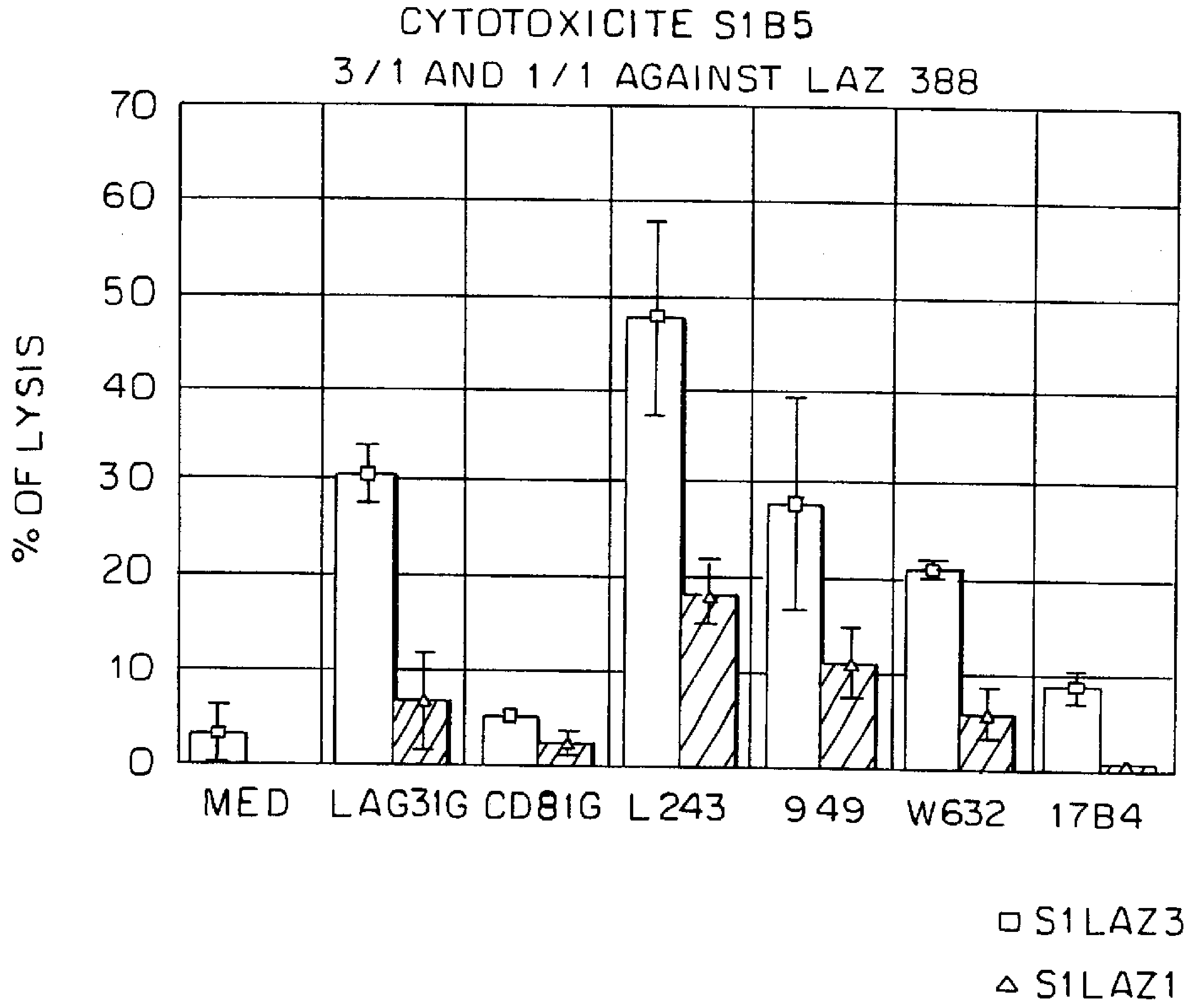
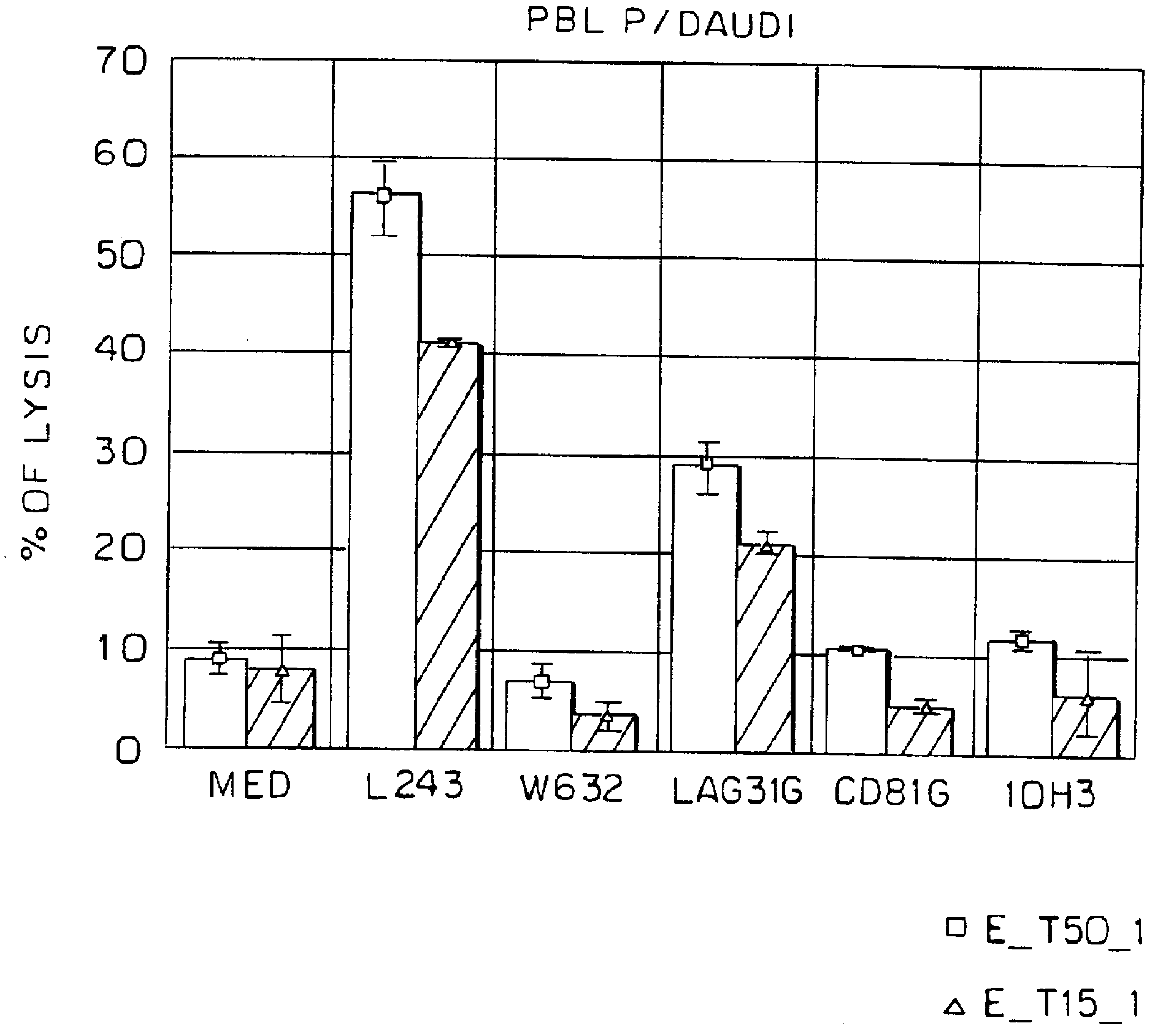

CYTOTOXICITE S1B5
3/1 AND 1/1 AGAINST LAZ 388
70
60
50
40
30
20
10
0
% OF LYSIS
MED
LAG3IG
CD8IG
L243
949
W632
17B4
□ S1LAZ3
△ S1LAZ1

FIG. 15


PBL P/DAUDI
70
60
50
40
30
20
10
0
% OF LYSIS
MED
L243
W632
LAG3IG
CD8IG
10H3
□ E_T50_1
△ E_T15_1

SOLUBLE POLYPEPTIDE FRACTIONS OF THE LAG-3 PROTEIN, PRODUCTION METHOD, THERAPEUTIC COMPOSITION, ANTI-IDIOTYPE ANTIBODIES

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a §371 application of PCT/FR95/00593, filed May 5, 1995.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to soluble forms derived from the LAG-3 membrane protein which are useful as immunosuppressants, as well as antibodies capable of preventing the specific binding of the LAG-3 protein to MHC (major histocompatibility complex) Class II molecules as immunostimulants.

2. Description of the Related Art

In WO-A 91/10682, a protein designated LAG-3 has been described.

The LAG-3 protein is a protein selectively expressed by NK cells and activated T lymphocytes. Similarity of the amino acid sequence, the comparative exon/intron organization and the chromosomal localization show that LAG-3 is related to CD4. The initial characterization of the LAG-3 gene has been described by TRIEBEL et al. (1).

The corresponding DNA codes for a type I transmembrane protein of 498 amino acids containing 4 extra-cellular sequences of the immunoglobulin type. LAG-3 is a member of the immunoglobulin superfamily.

The mature protein comprises 476 amino acids (SEQ ID No. 1) with a theoretical molecular weight of 52 kD. The extracellular region contains 8 cysteine residues and 4 potential N-glycosylation sites. By Western blot analysis, it was shown that LAG-3 inside PRA-blasts or activated NK cells has an apparent mass Mr of 70,000. After treatment with N-glycosidase F, a reduction in size to 60 kD was obtained, thereby demonstrating that native LAG-3 is glycosylated. Fuller details are described in WO-A 91/10682.

BAIXERAS et al., in J. Exp. Med. 176, 327–337 (2), have, in addition, described their finding that rosette formation between cells transfected with LAG-3 (expressing LAG-3 at their surface) and B lymphocytes expressing MHC Class II was specifically dependent on LAG-3/MHC Class II interaction.

Surprisingly, this ligand for MHC Class II was detected with higher levels on activated CD8$^+$ lymphocytes (MHC Class I-restricted) than on activated CD4$^+$ lymphocytes. In vivo, only a few disseminated LAG-3$^+$ cells (MHC Class II-restricted) were to be found in non-hyperplastic lymphoid tissue comprising the primary lymphoid organs, that is to say thymus and bone marrow. LAG-3$^+$ cells were to be found in hyperplastic lymphoid nodules and tonsils, as well as among peripheral blood mononuclear cells (PBMC) of patients receiving injections of high doses of IL-2.

These observations confirm that LAG-3 is an activation antigen in contrast to CD4 expressed in a subpopulation of resting lymphocytes and other cell types, in particular macrophages.

The MHC comprises Class I and Class II molecules which are membrane glycoproteins which present fragments of protein antigens to the T lymphocyte receptors (TCR). Class I molecules are responsible for the presentation to CD8$^+$ cytotoxic cells of peptides derived in large part from endogenously synthesized proteins, while Class II molecules present to CD4$^+$ helper lymphocytes peptides originating in the first place from foreign proteins which have entered the endocytic, that is to say exogenous, pathway. T helper lymphocytes regulate and amplify the immune response, while cytotoxic lymphocytes are needed to destroy cells irrespective of the tissues expressing "non-self" antigens, for example viral antigens. The mechanism of recognition involves intercellular signals leading to an effective activity of T lymphocytes.

It is apparent that, to initiate an immune response mediated by T (CD4$^+$) lymphocytes, the foreign antigens must be captured and internalized in the form of peptides by specialized cells, the antigen presenting cells (APC). The resulting antigenic peptides are reexpressed at the surface of the antigen presenting cells, where they are combined with MHC Class II molecules. This MHC Class **[**I**]** *II*/peptide complex is specifically recognized by the T lymphocyte receptor, resulting in an activation of the T helper lymphocytes.

Moreover, animal models created by recombination techniques have made it possible to emphasize the part played in vivo by MHC Class II molecules and their ligands.

Thus, mice deficient in MHC Class II molecules (3) and possessing almost no peripheral CD4$^+$ T lymphocytes and having only a few immature CD4$^+$ lymphocytes in the thymus have proved to be completely incapable of responding to T-dependent antigens.

CD4$^{-}/^{-}$ mutant mice (4) have a substantially decreased T lymphocyte activity but show normal development and function of the CD8$^+$ T lymphocytes, demonstrating that the expression of CD4 on the daughter cells and CD4$^+$ CD8$^+$ thymocytes is not obligatory for the development. Compared to normal mice, these CD4-deficient mice have a large amount of CD4$^-$ CD8$^-$ cells.

These doubly negative cells are restricted to MHC Class II and capable of recognizing the antigen.

When they are infected with Leishmania, these mice show a population of functional T helper lymphocytes despite the absence of CD4. These cells are restrictive to MHC Class II and produce interferon-γ when they are activated by the antigen. This indicates that the lineage of the T lymphocytes and their peripheral function need not necessarily depend on the function of CD4.

It is now recognized that the proteins encoded by MHC Class II region are involved in many aspects of immune recognition, including the interaction between different lymphoid cells such as lymphocytes and antigen presenting cells. Different observations have also shown that other mechanisms which do not take place via CD4 participate in the effector function of T helper lymphocytes.

These different observations underline the pivotal role played by MHC Class II and its ligands in the immune system.

Moreover, the importance is known of chimeric molecules composed of the extracytoplasmic domain of proteins capable of binding to ligands and a constant region of human immunoglobulin (Ig) chains for obtaining soluble forms of proteins and of cell receptors which are useful, in particular, as therapeutic agents.

Thus, soluble forms of CD4 have proven their efficacy in inhibiting an HIV infection in vitro in a dose-dependent manner.

Nevertheless, clinical trials with soluble CD4 molecules, in particular of CD4-Ig, have not enabled a significant decrease in viral titres to be demonstrated. Transgenic mice expressing up to 20 μg/ml of soluble CD4 in their serum were created. These mice showed no difference as regards their immune function relative to control mice. Hitherto, no direct biding to MHC Class II of molecules derived from CD4 has been reported. This strongly suggests that soluble CD4 molecules do not interact in vivo with MHC Class II molecules.

SUMMARY OF THE INVENTION

Surprisingly, the authors of the present invention have shown that soluble molecules containing different fragments of the extracytoplasmic domain of the LAG-3 protein were capable of binding to MHC Class II molecules and of having an immunosuppressant action.

The extracytoplasmic region of LAG-3 represented by the sequence SEQ ID No. 1 comprises the domains D1, D2, D3 and D4 extending from amino acids 1 to 159, 160 to 239, 240 to 330 and 331 to 412, respectively.

Thus, the subject of the invention is a soluble polypeptide fraction consisting of all or part of at least one of the 4 immunoglobulin type extracellular domains of the LAG-3 protein (amino acid 1 to 159, 160 to 239, 240 to 330 and 331 to 412 of the sequence SEQ ID No. 1), or of a peptide sequence derived from these domains by replacement, addition and/or deletion of one or more amino acids, and which possesses a specificity at least equal to or greater than that of LAG-3 for its ligand.

The present invention encompasses, in particular, soluble polypeptide fractions having a sequence derived from the native LAG-3 sequence originating from the well-known phenomenon of polytypy.

The soluble polypeptide fraction is characterized in that it comprises the peptide region of LAG-3 responsible for the affinity of LAG-3 for MHC Class II molecules.

The soluble polypeptide fraction comprises, in particular, a peptide sequence derived from these domains by replacement, addition and/or deletion of one or more amino acids, and which possess a specificity equal to or greater than that of LAG-3 for its ligand, for example the whole of the first two immunoglobulin type domains of LAG-3, or the 4 immunoglobulin type domains of the extracytoplasmic domain of LAG-3.

Advantageously, the soluble polypeptide fraction is comprised of all or part of at least one of the four immunoglobulin type extracellular domains of the LAG-3 protein (amino acid 1 to [149, 150] *159, 160* to 239, 240 to 330 and 331 to 412 of sequence SEQ ID No. 1) comprising one or more of the arginine (Arg) rests at the positions 73, 75 and 76 of sequence SEQ ID No. 1 substituted with glutamic acid (Glu).

Preferably, the soluble polypeptide fraction comprises a loop in which the average position of the atoms forming the basic linkage arrangement is given by the position of amino acids 46 to 77 (SEQ ID No. 1) appearing in Table 1 or Table 2 or differs therefrom by not more than 5%.

The soluble polypeptide fraction advantageously comprises, in addition, the second immunoglobulin type extracellular domain (D2) of LAG-3 (amino acids [150] *160* to [241] *239*).

Advantageously, the soluble polypeptide fraction comprises, besides the peptide sequence of LAG-3 as defined above, a supplementary peptide sequence at its C-terminal and/or N-terminal end, so as to constitute a fusion protein. The term "fusion protein" means a portion of any protein permitting modification of the physicochemical features of the subfragments of the extracytoplasmic domain of the LAG-3 protein. Examples of such fusion proteins contain fragments of the extracytoplasmic domain of LAG-3 as are defined above, bound to the heavy chain —CH2—CH3 junction region of a human immunoglobulin, preferably an isotype IgG4 immunoglobulin.

Such fusion proteins may be dimeric or monomeric. These fusion proteins may be obtained by recombination techniques well known to a person skilled in the art, for example a technique such as that described by Traunecker et al. (5).

Generally speaking, the method of production of these fusion proteins comprising an immunoglobulin region fused with a peptide sequence of LAG-3 as defined above consists in inserting into a vector the fragments of cDNA coding for the polypeptide regions corresponding to LAG-3 or derived from LAG-3, where appropriate after amplification by PCR, and the cDNA coding for the relevant region of the immunoglobulin, this cDNA being fused with cDNA coding for the corresponding polypeptide regions or derivatives of LAG-3, and in expressing after transfection the fragments cDNA in an expression system, in particular mammalian cells, for example hamster ovary cells.

The fusion proteins according to the invention may also be obtained by cleavage of a LAG-3/ Ig conjugate constructed so as to contain a suitable cleavage site.

The subject of the invention is also a therapeutic composition having immunosuppressant activity comprising a soluble polypeptide fraction according to the invention. This composition will be useful for treating pathologies requiring immunosuppression, for example autoimmune diseases.

The subject of the invention is also the use of antibodies directed against LAG-3 or soluble polypeptide fractions derived from LAG-3 as are defined above, or fragments of such antibodies, in particular the Fab, Fab' and $F(ab')_2$ fragments, for the preparation of a therapeutic composition having immunostimulatory activity. "Immunostimulatory" means a molecular entity capable of stimulating the maturation, differentiation, proliferation and/or function of cells expressing LAG-3, that is to say T lymphocytes or active NK cells. The anti-LAG-3 antibodies may be used as potentiators of vaccines or immunostimulants in immunosuppressed patients, such as patients infected with HIV or treated with immunosuppressant substances, or be used to stimulate the immune system by elimination of self cells displaying abnormal behaviour, for example cancer cells.

Immunostimulatory activity of anti-LAG-3 anti-bodies is surprising, inasmuch as anti-CD4 antibodies have an immunosuppressant action.

Such antibodies may be polyclonal or monoclonal; however, monoclonal antibodies are preferred. The polyclonal antibodies may be prepared according to well-known methods, such as that described by BENEDICT A. A. et al. (6). Monoclonal antibodies are preferred, on account of the fact that they are specific for a single epitope and yield results with better reproducibility. Methods of production of monoclonal antibodies are well known from the prior art, especially the one described by KOHLER and MILSTEIN. This method, together with variants thereof, are described by YELTON et al. (7).

The subject of the invention is also anti-idiotype antibodies directed against the antibodies according to the invention, which contain the internal image of LAG-3 and are consequently capable of binding to MHC Class II. Such antibodies may be used, in particular, as immunosuppressants, and, for example, in autoimmune pathologies.

The therapeutic compositions according to the present invention comprise soluble LAG-3 proteins or antibodies as are defined above, as well as a pharmaceutically acceptable vehicle. These compositions may be formulated according to the usual techniques. The vehicle can vary in form in accordance with the chosen administration route: oral, parenteral, sublingual, rectal or nasal.

For the compositions for parenteral administration, the vehicle will generally comprise sterile water as well as other possible ingredients promoting the solubility of the composition or its ability to be stored. The parenteral administration routes can consist of intravenous, intramuscular or subcutaneous injections.

The therapeutic composition can be of the sustained-release type, in particular for long-term treatments, for example in autoimmune diseases. The dose to be administered depends on the subject to be treated, in particular on the capacity of his/her immune system to achieve the desired degree of protection. The precise amounts of active ingredient to be administered may be readily determined by the practitioner who will initiate the treatment.

The therapeutic **[**.compositions**]** *compositions* according to the invention can comprise, in addition to soluble LAG-3 or the antibodies according to the invention, another active ingredient, where **[**apprto LAG-3 or to anmical**]** *appropriate, bound via a chemical* bond to LAG-3 or to an antibody according to the invention. As an example, there may be mentioned soluble LAG-3 proteins according to the invention fused to a toxin, for example ricin or diphtheria toxoid, capable of binding to MHC Class II molecules and of killing the target cells, for example leukaemic or melanoma cells, or fused to a radioisotope.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows a comparison of the proliferation of T cells incubated with F(ab) fragments of 17B4 to the proliferation of T cells incubated with intact 17B4 monoclonal antibody.

FIG. **2** shows the proliferation of Clone 28 in response to tetanus toxoid when co-cultured with 17B4 or control antibody 10H3.

FIG. **3** shows the expression vector pCDM7 used for manufacturing the recombinant LAG-3 proteins and a recombinant CD8 immunoadhesin control.

FIG. **4** shows the pCLH3 AXS V2 DHFR hα IVS vector used to express amplified LAG-3 sequences.

FIG. **5**A shows the inhibition of MHC Class II interaction with LAG-3 by recombinant LAG-3 D1–D4 and FIG. **5**B shows the potential inhibition of MHC Class II interaction with CD4.

FIG. **6** shows the inhibition of Clone 28 proliferation by recombinant LAG-3 D1–D4.

FIG. **7** shows the inhibition of Clone 154 proliferation by LAG-3 Ig.

FIG. **8** shows the binding of LAG-3 to B cell lines expressing MHC class II haplotypes or human class II-transfected mouse cells.

FIG. **9** shows the binding of LAG-3 Ig to MHC Class II expressing Daudi cells.

FIG. **10** shows that preincubation of HLA class II expressing cells with 17B4 inhibits LAG-3 Ig binding.

FIGS. **11**A–**11**C show the inhibition of clone T154 proliferation by crosslinked LAG-3 Ig.

FIG. **12** compares inhibition of T cell proliferation by anti-Class II antibodies to inhibition of T cell proliferation by LAG-3 Ig.

FIGS. **13**A–**13**D show that T cell proliferation in response to OKT3 (FIG. **13**A), lectins (FIG. **13**B) and low-concentration IL$_2$ (FIG. **13**C) is inhibited by LAG-3 Ig but proliferation in response to high-concentration IL$_2$ is not inhibited by LAG-3 Ig (FIG. **13**D).

FIG. **14** shows clone S1B5 cytotoxicity towards Epstein-Barr virus transformed human B cells.

FIG. **15** shows peripheral blood lymphocyte cytotoxicity towards HLA Class I$^-$ Daudi cells.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The examples which follow, together with the attached reference figures, will illustrate the invention in greater detail.

EXAMPLE 1

Proliferation of active T lymphocyte lines in the presence of anti-LAG-3 monoclonal antibodies

The anti-LAG-3 monoclonal antibodies used were 17B4, described in BAIXERAS et al. (2) and deposited at the CNCM under No. I-1240 on Jul. 10, 1992, and 11E3, described in HUARD et al. (8).

These antibodies belong to the isotype IgG1. These antibodies were tested for their biological effects on activated T lymphocytes, stimulated by specific antigenic peptides or processed antigens presented by MHC Class II molecules expressed by autologous antigen presenting cells, expressing LAG-3.

An anti-CD48 monoclonal antibody designated 10 H3 was used as irrelevant IgG1 antibody (negative control).

The saturating concentrations of anti-LAG-3 and anti-CD48 antibodies were determined by immunofluorescence on PHA (phytohaemagglutinin)-blasts and cell lines transformed by Epstein-Barr virus (EBV). In the proliferation tests, the monoclonal antibodies were added in the proportion of 5 times the saturating concentration.

The T lymphocyte lines used were, on the one hand the clone 154 derived from peripheral blood lymphocytes, raised against a peptide mimicking an influenza haemagglutinin (HA) fragment having an amino acid sequence extending from amino acid 306 to 329 (p20 peptide), and on the other hand the clone 28, a T lymphocyte clone derived from peripheral lymphocytes of a single human donor, raised against diphtheria toxoid (DT). The antigen presenting cells (APC) corresponding to clone 154 were EBV-transformed B lymphocytes of the same donor (DR3/DR11) as T 154. The antigen presenting cells corresponding to clone 28 were EBV-transformed B lymphocytes of the same donor. This clone was restricted to HLA DR7.

For clone 154, the APC (5×10^6) were incubated at 37° C. for one and a half hours with variable doses of the p20 peptide, then washed and irradiated (10,000 rad). The cells were plated out on 96-well microtitration plates at the same time as the clone 154 cells (0.5×10^5 to 10×10^5 cells/ml) in a 3:1 ratio. For clone 28, the responding cells/stimulating cells ratio was 1.

The HLA DR7/EBV APC cells were either treated with mitomycin or irradiated, then added to the T lymphocytes in the presence of DT (which remained in the culture). The final concentration of clone 28 cells was 100,000 cells/ml.

[^{3}H]Thymidine (1 μCi/well) was added at varying time intervals from day 2 to day 10 of culture.

Each experiment was carried out in triplicate.

The results were expressed as the mean cpm and after subtraction of the cpm found in the negative control (T lymphocytes cocultured with APC unladen with immunogens). The proliferation tests were carried out on 96-well plates. The absorption of tritiated thymidine in the individual 200 μl wells was measured after adding 1 μCi of thymidine for the last 18 hours of culture. The results were expressed in the form of the mean of 3 tests. The standard deviation was usually less than 12% (a little more in the case of very low cpm measurements). Moreover, mixed culture (clone 154/APC) supernatants were combined, filtered through 0.22 μm membranes, divided into samples and frozen at −20° C. until the time of titration using commercial immunoassay kits: Immunotech IL-2 and INF-α titration kit, Genzyme IFN-γ kit and Cayman Chemicals IL-4 kit.

A dose determination study was carried out to establish the proliferation profiles of clone 154 brought into contact with the p20 specific antigen at varying concentrations and in the presence or absence of anti-LAG-3 monoclonal antibodies or irrelevant monoclonal antibodies (negative control).

The individual results of 16 separate tests showed that, irrespective of the concentration of added antigen, the initial point up to the peak of proliferation was not modified, but a significant prolongation of the proliferation of T lymphocytes incubated with the anti-LAG-3 monoclonal antibodies was observed systematically. Fab fragments of the monoclonal antibody 17B4 were prepared and used in a test of proliferation of clone 154. The proliferation profile of T lymphocytes activated by the antigen with the 17B4 Fab fragments (15 μg/ml) was similar to that of cells incubated in the presence of whole 17B4 monoclonal antibody (40 μg/ml) (FIG. **1**). These results show that the observed biological effects are not attributable to a non-specific reaction induced by the Fc region of the anti-LAG-3 monoclonal antibodies.

Similar results were obtained with the 11E3 anti-LAG-3 monoclonal antibodies.

Clone 28 was also stimulated with the antigen (tetanus toxoid 10 μg/ml) in the presence of 17B4 monoclonal antibodies after coculture with the corresponding APC in the presence of DT. The results are shown in FIG. **2**.

The effects of the anti-LAG-3 monoclonal antibodies observed with clone 28, namely the prolongation of proliferation, are similar to those observed with clone 154.

Tests were carried out designed to measure the miscellaneous cellular events occurring after the antigenic stimulation of clone 154 cells incubated in the presence of anti-LAG-3 monoclonal antibodies.

The cells were harvested during conventional antigenic stimulation of clone 154 in the presence of anti-LAG-3 or anti-CD48 monoclonal antibodies or in the absence of antibodies, and tested for the expression of LAG-3 and CD25 transmembrane receptors, and samples of culture supernatants were collected at different time intervals after stimulation and tested for the presence of IFN-γ, TNF-α, IL-4 and IL-2.

Two-colour direct immunofluorescence tests (anti-CD3 monoclonal antibodies and anti-CD25 monoclonal antibodies) showed that IL-2 receptors were weakly but significantly increased 5 days after the antigenic stimulation. Similar tests with anti-CD3 and 11E3 (anti-LAG-3) monoclonal antibodies showed that LAG-3 was over-expressed from the day following activation onwards. In addition, the secretion of IL-2, IL-4, IFN-γ and TNF-α was also modulated by incubation with anti-LAG-3 monoclonal antibodies, thus showing that different cellular events are modified by the presence of anti-LAG-3 monoclonal antibodies and that some events already take place 24 hours after stimulation.

These results show indirectly that LAG-3 plays a regulatory role for CD4$^+$ cells. The fact that anti-LAG-3 monoclonal antibodies increase proliferation, and hence act as immunopotentiators, suggest that LAG-3 is involved in the "deactivation" of CD4$^+$ T lymphocytes with a negative role of LAG-3 on the antigen-dependent stimulation.

EXAMPLE 2

Transient expression of LAG-3 fusion proteins

Soluble proteins derived from LAG-3 were obtained by a recombinant DNA technique using suitable vectors comprising DNA coding for LAG-3 and DNA coding for an immunoglobulin fragment. The transient expression system consisted of transfected Cos cells. This system makes it possible to produce several mg of recombinant fusion proteins. Recombinant DNA techniques were carried out as described by MANIATIS et al. (22). The modifications were made as recommended by the manufacturer.

Construction of LAG-3 D1–D4 Ig and LAG-3 D1D2 Ig

Fragments coding for the D1D2 or D1–D4 regions were amplified (30 cycles) from a fragment of cDNA (FDC sequence) encompassing LAG-3 cDNA (TRIEBEL et al. (1)), using Taq polymerase free from 5'-endonuclease activity and relatively resistant to an exposure to very high temperature; the amplification was followed by a denaturation at 98° C. (with a Perkin Elmer Cetus "DNA thermal cycle"). Specific primers were used as recorded in the table below.

The resulting amplified fragments (739 bp and 1312 bp for LAG-3 D1–D2 and LAG-3 D1–D4, respectively) were inserted into a pBS plasmid (Stratagene).

Inserts were prepared after digestion with XhoI and BglII and introduced into the XhoI/BamHI sites of the vector pCDM7-CD8-IgG1 (pCDM7 being derived from pCDM8 marketed by Stratagene), as illustrated in FIG. **3**, so as to exchange the DNA sequences coding for CD8 for those coding for the subfragments of LAG-3. The resulting expression vectors contained the sequences coding for D1D2 or D1–D4 fused to the DNA sequences coding for the —CH_2—CH_3 junction region of a human IgG1 chain.

TABLE 3

Primers used to amplify LAG-3 DNA sequences by PCR

Primers used for amplification of the DNA	Resulting encoded subfragment fused with a subfragment Ig
Primer (5') 5' GCGCCTCGAGGCCCAGACCATAGGAGAGATGT 3' (SEQ ID NO: 2) coupling site untranslated 5' sequences start of translation	LAG-3 D1D2 from the leader sequence to amino acid[241] *239*
Primer (3') 5' GCGCAGATCTCTCCAGACCCAGAACAGTGAGGTTATACAT 3' (SEQ ID NO: 3) BglII coupling site End of D2	
Primer (5') identical to LAG-3 D1D2	LAG-3 D1-D4 from the leader
Primer (3') 5' GCGCAGATCTACCTGGGCTAGACAGCTCTGTGAA 3' (SEQ ID NO: 4) BglII coupling site End of D4	sequence to amino acid 412

CDM7 is a eukaryotic expression vector derived from the vectors developed by SEED et al. (10) for the cloning of DNA and its expression in E. coli and eukaryotic cells. CDM7 possesses the following features: (i) the human cytomegalovirus promoter for transient expression in mammalian cells; (ii) a viral origin of SV40 for an autosomal replication of mammalian cells expressing T antigen; (iii) πVX (type Col E1) as plasmid origin for a high copy number; (iv) a Sup F selection for resistance to ampicillin and tetracycline in Tet^{amb} and Amp^{amb} E. coli strains; (v) an origin of replication of M13 for the release of a single strand; (vi) a T7 RNA promoter; and (vii) a polylinker for an efficient cloning of heterologous DNA.

Transient expression in COS cells

Cos cells (5×10^{6}) were transfected with 30 μg of DNA of suitable expression vectors (coding for either LAG-3 D1D2 Ig, or LAG-3 D1–D4 Ig, or CD8 Ig) by electroporation (200 V, 1500 μF, 30–40 msec) using a Celliect apparatus (Eurogentech, Liège, BE). The cells were plated out again and cultured on a medium containing 5% of foetal calf serum. The supernatants were withdrawn 6 days after transfection.

The synthesis of the resulting fusion proteins was analysed from the supernatants as well as from cell extracts of transfected cells, by Western blot analysis with the 17B4 monoclonal antibodies. Immunoreactive materials were observed in the supernatant of cells transfected with DNA coding for LAG-3 D1D2 Ig or LAG-3 D1–D4 Ig.

Concomitantly, a recombinant CD8 immunoadhesin (CD8 Ig) was obtained as negative control using the same expression system and the expression vector pCDM7-CD8 (FIG. **3**).

The recombinant proteins LAG-3 D1D2 Ig. LAG-3 D1–D4 Ig and CD8 Ig were purified by means of the standard method on protein A-Sepharose. The resulting material was analysed by SDS-PAGE, followed by Coomassie staining or a Western blot analysis using anti-human Ig antibody.

EXAMPLE 3

Production of soluble subfragments of LAG-3

In order to produce large amounts of recombinant proteins, a stable expression system consisting of transfected mammalian cells was developed. The host cells are anchorage-dependent hamster ovary (CHO) cells isolated from CHO cells deficient in dihydrofolate reductase (DHFR) and consequently necessitating glycine, a purine and thymidine for their growth. The pivotal role of DHFR in the synthesis of nucleic acid precursors, combined with the sensitivity of DHFR-deficient cells with respect to tetrahydrofolate analogues such as methotrexate (MTX), has two major advantages. Transfection of these cells with expression vectors containing the DHFR gene permits the secretion of recombinant DHFR-resistant clones, and the culturing of these cells on selective media containing increasing amounts of MTX results in amplification of the DHFR gene and the DNA associated therewith.

Construction of LAG-3 D1, LAG-3 D1D2, LAG-3 D1–D4

Fragments of DNA coding for the D1, D1D2 or D1–D4 regions were amplified using a PCR method identical to the one described previously, using the primers specified in the table below.

TABLE 4

Primers used for amplifying LAG-3 DNA sequences by PCR

Primers used for amplification of the DNA	Resulting encoded subfragment
Primer (5') 5' CGCCGTCGACCGCTGCCCAGACCATAGGAGAGATGTG 3' (SEQ ID NO:5) SalI coup- untranslated start of ling site 5' sequences translation Primer (3') 5' GCGCGTCGACTTAACCCAGAACAGTGAGGTTATAC 3' (SEQ ID NO: 6) SalI coup- End of D1 ling site	LAG-3 D1 from the leader sequence to amino acid [149]*159*
Primer (5') identical to LAG-3 D1 Primer (3') 5' GCGCGTCGACTTAACCCAGAACAGTGAGGTTATAC 3' (SEQ ID NO: 7) SalI[II] coup- End of D2 ling site	LAG-3 D1D2 from the leader sequence to amino acid 239
[Primer (3') 5' GCGCGTCGACTTAACCCAGAACAGTGAGGTTATAC 3' (SEQ ID NO: 6) SalI coup- End of D1 ling site]	[amino acid 149]
Primer (5') identical to LAG-3 D1 Primer (3') 5' GCGCGTCGACTTAACCCTGGGCTAGACAGCTCTCTGTG 3' (SEQ ID NO:8) SalI coup- End of D4 ling site	LAG-3 D1-D4 from the leader sequence to amino acid 412

The resulting amplified fragments were digested with SalI and inserted into the SalI site of pUC 18 (Stratagene).

The amplified sequences were verified, and the inserts subcloned into the expression vector pCLH3 AXS V2 DHFR hα IVS as described by COLE et al. (Biotechnology 11, 1014–1024, 1993) (FIG. **4**).

This vector is a eukaryotic expression vector which is multifunctional for the expression [C]cDNA and its amplification in eukaryotic cells. It possesses the following features: (i) the murine promoter of the metallothionein-1 gene and a polyadenylation sequence SV 40 (comprising a donor-acceptor splicing site) to bring about transcription of the gene of interest, (ii) a human intervening sequence A containing the donor-acceptor splicing site of the gene for the subunit of α glycoprotein for obtaining high levels of transcription of cDNA, (iii) the pML sequence containing the origin of replication of pBR322 and a gene for resistance to [aampicillin] *ampicillin* for bacterial amplification, and (iv) a DHFR transcription unit of SV 40 to bring about transcription of the sequences used for selection and amplification of the transfectants.

Stable expression in CHO cells

The expression vectors coding for LAG-3 D1, LAG-3 D1D2 and LAG-3 D1–D4 were used to transfect CHO DUKX cells, and these cells were cultured on a selective medium. Cells capable of multiplying under these conditions were combined and cultured on a medium containing increasing amounts of MTX. Levels of expression were measured by Western blot analysis using the 17B4 monoclonal antibody. Clones producing high levels of recombinant soluble molecules derived from LAG-3 were propagated in bioreactors, and the material derived from LAG-3 was purified by ion exchange chromatography and immunoaffinity.

Western blot analyses revealed, in supernatants of cells transfected with expression vectors coding for LAG-3 D1, LAG-3 D1D2 and LAG-3 D1–D4, bands with apparent Mr values of 15 to 18 kD, 34–36 kD (doublets) and 55 kD (2 possible bands). The respective Mr values of these immunoreactive materials corresponded to the expected Mr values of glycosylated LAG-3 D1 Ig (139 amino acids and a putative N-glycosylation site), glycosylated LAG-3 D1D2 Ig (239 amino acids containing 3 glycosylation sites) and glycosylated LAG-3 D1–D4 (412 amino acids containing 4 glycosylation sites).

EXAMPLE 4

Specific binding of LAG-3 Ig to cells expressing MHC Class II

The reactivity of the monoclonal antibodies and of LAG-3 D1–D4 Ig was studied by indirect immunofluorescence. Target cells (4×10^5) were incubated for 30 minutes at 4° C. in the presence of LAG-3 D1–D4 Ig, CD8 Ig, a murine monoclonal antibody, (949) anti-human MHC Class II (DR, DP, DQ) conjugated to FITC (isothiocyanate fluoride) from a Coulter clone, or murine Ig-FITC; an irrelevant immunoglobulin G conjugated to FITC. The cells were washed and incubated at 4° C. for 30 minutes with either a goat anti-human Ig polyclonal $F(ab')_2$ conjugated to fluorescein or a goat anti-mouse Ig polyclonal antibody conjugated to fluorescein (Coulter clone).

To confirm the LAG-3/MHC Class II binding, LAG-3 D1–D4 Ig was incubated with MHC Class II-positive or -negative cells. Four B lymphocyte lines expressing MHC Class II(L31, Phil EBV, Raji, Sanchez and Personnaz) were treated with anti-Class II monoclonal antibody 949, or the supernatants for Cos cells transfected with DNA coding either for LAG-3 D1–D4 Ig or for CD8 Ig. The five cell lines expressing the different haplotypes of MHC Class II molecules were recognized by LAG-3 Ig in the same way as by the anti-Class II monoclonal antibodies (positive control), while the supernatant containing CD8 Ig (negative control) did not bind to these cell lines, as could be expected. Four

MHC Class II-negative cell lines (CEM, RJ, HSB2, K562) were treated with the same reagents as above. None reacted, either with the anti-MHC Class II (negative control) or with LAG-3 D1–D4 Ig, showing that the binding of LAG-3 D1–D4 is specific to MHC Class II molecules.

Further experiments were carried out using (i) mouse fibroblasts transfected or otherwise with genes coding for human DR7 or human DP4, (ii) mouse cells expressing or otherwise MHC Class II molecules, (iii) activated human CD4$^+$ or CD8$^+$ cells, and (iv) T lymphocyte lines expressing the different haplotypes of MHC Class II molecules (FIG. **8**).

Unlike CD8 Ig, LAG-3 D1–D4 Ig binds to all cells expressing MHC Class II as efficiently as the anti-MHC Class II monoclonal antibody 949. LAG-3 D1–D4 Ig binds to all DR and DP haplotypes tested, to human MHC Class II molecules expressed by transfected mouse cells, to murine MHC Class II molecules and also to MHC Class II molecules expressed by CD4$^+$ or CD8$^+$ T lymphocytes.

These results represent for the first time proof that soluble molecules derived from a ligand for MHC Class II are capable of binding to cells expressing MHC Class II.

Similar experiments showed that LAG-3 D1D2 bound to cells expressing MHC Class II in as specific a manner and with the same efficiency as LAG-3 D1–D4.

Binding activity of LAG-3Ig and cellular distribution of ligands for LAG-3Ig

The capacity of this immunoadhesin to bind to cell ligands is measured using a fluorescein-labeled goat serum directed against human immunoglobulins.

In these experiments, the target cells are first incubated with a human monoclonal antibody or an immunoadhesin for 30 min at 4° C. in RPMI 1640 containing 10% of FCS (foetal calf serum). The cells are then incubated with an FITC-labelled goat anti-mouse immunoglobulin serum (Coulter) for the murine monoclonal antibodies or with an FITC-labelled goat anti-human immunoglobulin serum (Tago) for the immunoadhesins. The fluorescence is measured after two washes, analyzing 3,000 cells with an Elite cytometer (Coultronics, Hialeah, Fla.). FIG. **9** shows the degrees of binding of LAG-3Ig, CD8Ig, antibody 949 or antibody OKT3 (anti-CD3, ATCC), represented by the number of cells counted as a function of the logarithm of the measured fluorescence intensity.

LAG-3Ig binds to mouse fibroblasts transfected for the gene for the HLA DR$_4$ molecule, and does not bind to untransfected cells. CD8Ig is incapable of binding to HLA DR$_4$$^+$ fibroblasts under the same conditions.

The cellular distribution of the ligands for LAG-3Ig was evaluated on a cell population sample by immunofluorescence.

LAG-3Ig is visualized on all positive Class II cells tested, including B cell lines transformed by Epstein-Barr virus (derived from genetically unrelated donors, including 10 homozygous lines of DR$_1$ to DR$_{10}$ typing), as well as on activated T and NK cells.

FIG. **9** shows, by way of example, the binding of LAG-3Ig to Daudi cells which are positive for Class II antigens.

The mean fluorescence intensity with LAG-3Ig is similar to that observed with antibody 949 which is specific for Class II antigens. The binding of LAG-3Ig to DR$_4$ (FIG. **9**). DR$_2$, DR$_7$ or DPw4 (not shown) expressed at the surface of mouse fibroblasts is, in contrast, weaker than that observed for antibody 949.

No binding is detected on cell lines which are negative for Class II antigens of T origin (peripheral blood T cells, CEM, HSB2, REX lines), of B origin (RJ 2.2.5 line) or of non-lymphoid origin (human lines, K562 of erythromyoloid origin and line originating from melanoma cells (not shown)).

Moreover, LAG-3Ig binds to xenogeneic Class II molecules of the MHC, such as the antigens expressed by mouse lymphoid A 20 and the monkey Classes II expressed by phytohaemagglutinin-stimulated blasts (data not shown).

The specificity of binding of LAG-3Ig was also verified using the monoclonal antibodies 17B4, whose capacity to block LAG-3/MHC Class II interactions in cell adhesion tests was demonstrated beforehand (FIG. **10**).

In these experiments, the LAG-3Ig molecules are preincubated for 30 minutes at 4° C. either with medium alone, or with 17B4 (1 mg/ml), or with OKT3 (1 mg/ml), before being brought into contact with Daudi cells.

FIG. **10** shows that a preincubation of LAG-3Ig with 17B4 inhibits the binding to Class II$^+$ cells, whereas no inhibition is detected with the OKT3 control.

EXAMPLE 5

Inhibition of LAG-3/MHC Class II interaction by soluble fragments of LAG-3

The inhibition of LAG-3/MHC Class II interaction by the soluble fragments of LAG-3 may be observed directly in relation to the binding of LAG-3Ig by Class II MHC molecules, by competitive experiments with the soluble fragments.

To verify whether the soluble LAG-3D$_1$D$_2$ fragments produced by CHO cells could displace the binding of immunoadhesins derived from LAG-3, the following tests were carried out:

Daudi cells are incubated with soluble LAG3-D$_1$D$_2$ fragments so as to permit the binding of these molecules to the MHC Class II antigens expressed at the surface of the Daudi cells.

In a second step, the cells are incubated in the presence of LAG-3D$_1$D$_4$Ig in dimeric form or LAG-3D$_1$D$_2$Ig in monomeric form.

The binding of these immunoadhesins derived from LAG-3 is measured using a goat anti-human Ig F(ab')$_2$ conjugated to fluorescein (GAH-FITC).

The control groups are represented by Daudi cells incubated with dimeric LAG-3D$_1$D$_4$Ig or monomeric LAG-3D$_1$D$_2$Ig without preincubation with the soluble LAG-3D1D2 fragments.

The results are recorded in Table 5, which shows that the soluble LAG-3D$_1$D$_2$ fragments are capable of displacing the immunoadhesins derived from LAG-3 in **[**monoor**]** *mono or* dimeric form.

TABLE 5

Reactants	Detection	Mean Fluorescence	Conclusion
—	GAH-FITC	0.3	GAH does not interfere
Dimeric LAG-3D1D4Ig	GAH-FITC	20.8	The binding of CHO/LAG-3D1D2 inhibits the binding of dimeric LAG-3D1D4Ig (58%)
CHO/LAG-3D1D2, then dimeric LAG-3D1D4Ig	GAH-FITC	8.5	
Monomeric	GAH-FITC	62.5	The binding of

TABLE 5-continued

Reactants	Detection	Mean Fluor-escence	Conclusion
LAG-3D1D2Ig			
CHO/LAG-3D1D2 then monomeric LAG-3D1D2Ig	GAH-FITC	10.9	CHO/LAG-3D1D2 inhibits the binding of monomeric LAG-3D1D2Ig (27%)

These data confirm that the soluble LAG-3D1D2 fragments bind to MHC Class II molecules.

Inhibition of LAG-3/MHC Class II and CD4/MHC Class II interaction

Rosette formation between Cos cells transfected with wild-type LAG-3 and B lymphocytes transformed with EBV expressing MHC Class II molecules was demonstrated by BAIXERAS et al. (2). This interaction is inhibited both by anti-LAG-3 and anti-MHC Class II monoclonal antibodies.

The method described in this publication was modified by replacing the visualization and counting of Cos cells binding to B lymphocytes by counting the radioactivity remaining after incubation of ^{51}Cr-labelled B lymphocytes with Cos cells expressing LAG-3 (binding assay).

The possible inhibitory effects of soluble molecules derived from LAG-3 on LAG-3/MHC Class II interaction, and also on CD4/MHC Class II interaction, were studied.

Cos cells transfected with a suitable expression vector (coding for wild-type LAG-3 or for CD4). Two days later, the Cos cells were treated with trypsin and plated out again on the basis of 0.05x106 cells/well on a flat-bottomed 12-well tissue culture plates, 24 hours later. ^{51}Cr-labelled Daudi cells (5.5x10^6) were incubated on this monolayer of Cos cells (final vol.: 1 ml) for 1 hour. The target B cells were then aspirated off and the wells washed 5 to 7 times, gently adding 1 ml of medium dropwise. The edges of the wells were washed by suction using a Pasteur pipette. The remaining cells were lysed with 1 ml of PBS, 1% Triton for 15 minutes at 37° C. The lysates were centrifuged at 3000 rpm for 10 minutes, and 100 μl of the resulting supernatant were counted.

LAG-3 D1–D4 Ig was used to inhibit LAG-3/MHC Class II and CD4/MHC Class II interaction in the ^{51}Cr binding assay. Human CD8 Ig and IgG1 were tested in parallel and used as negative controls.

A significant inhibition of LAG-3/Class II interaction by LAG-3 D1–D4 Ig was detected (FIG. **5**A). However, the LAG-3/MHC Class II interaction can be partially and non-specifically inhibited by human CD8 Ig and IgG1. Moreover, LAG-3 Ig proved to be a potential inhibitor of CD4/Class II interaction (FIG. **5**B) under experimental conditions in which CD4/MHC Class II interaction was not modified by human CD8 Ig or IgG1. This suggests that LAG-3/Class II interaction is weaker than CD4/Class II interaction. These results represent the first proof of a possible competition of soluble molecules in an interaction of MHC Class II with its ligands.

EXAMPLE 6

Immunosuppressant activity of LAG-3 D1–D4 Ig

Functional tests were performed using the proliferation tests described above for the biological activity of the anti-LAG-3 monoclonal antibodies.

3 days and 5 days (D3 and D5) after antigenic stimulation, LAG-3 D1–D4 Ig showed a strong inhibition of the proliferation of clone 28, while human CD8 Ig and IgG had no effect (FIG. **6**). Similar experiments were carried out with clone 154 (FIG. **7**), and showed a partial inhibition in the presence of LAG-3 Ig. A control carried out with anti-LAG-3 monoclonal antibodies had the reverse effects, as observed previously.

A significant inhibition of the cell proliferation of cells incubated in the presence of LAG-3 D1–D4 Ig was also observed for clone 28.

These observations show that LAG-3 D1–D4 Ig is a potential immunosuppressant of the proliferation of T lymphocytes stimulated by an antigen, and indicate that LAG-3 might act as an “extinguisher” of the secondary immune response induced by activated CD4$^+$ T helper lymphocytes.

Role of LAG-3Ig in the negative regulation of the immune responses of T cells

To demonstrate that a soluble form of LAG-3, mimicking the functions of the membrane molecule, could inhibit the activation of CD$_4^+$ T clones stimulated by an antigen, the following tests were carried out on clone T154: the T cells are incubated beforehand with a saturating amount of LAG-3Ig (100 nM). The cells are then washed twice with cold RPMI and incubated with 10 μg/ml of goat antibodies directed against human immunoglobulins (Tago) at 4° C. for 30 minutes.

After two more washes, the cells are resuspended in RPMI containing 10% of foetal calf serum and incubated for 2 hours at 37° C. before adding the signal. To couple (“cross-link”) the monoclonal antibodies, a goat anti-mouse antibody at a concentration of 10 μg/ml (Tago) is used.

FIG. **11** depicts an experiment in which clone T154 has been preincubated with LAG-3Ig bound (“cross-linked”) to a second reactant (polyclonal serum specific for the constant region of human immunoglobulins). The degree of binding of LAG-3Ig to the cells is measured by immunofluorescence (FIG. **11**A). FIG. **11**B shows that a more than 50% inhibition of the proliferation of clone T154 is produced by LAG-3Ig. Under the same experimental conditions, no effect is observed with the control CD8Ig or with LAG-3Ig without “cross-linking” (not shown in the figure).

FIG. **11**C also shows that no effect is observed when LAG-3Ig is used to bind (“cross-link”) the MHC Class II molecules expressed by antigen-presenting B cells.

The possible effects of bound (“cross-linked”) anti-Class II monoclonal antibodies in relate to the proliferation of T cells were compared to those of LAG-3Ig. A weak inhibition (less than 50%) is observed with antibody 949 and antibody D1.12 (anti-DR) bound to a goat anti-mouse polyclonal serum (FIG. **12**). The inhibition of proliferation is hence epitope-dependent, the largest effect being obtained with the epitope of LAG-3 specific for the binding to **[**Classes**]** *Class* II.

The effects of LAG-3 Ig on the proliferation of T cells were also studied using different signals on another CD$_4^+$ T clone, clone TDEL specific for peptide 34–53 of the basic myelin protein.

An inhibition of proliferation is observed (n=2) when TDEL is stimulated with the antigen (not shown), with immobilized OKT3 (FIG. **13**A), with lectins (PHA+PMA) (FIG. **13**B) and with 5 IU/ml of IL$_2$ (FIG. **13**C). No inhibition is observed with 100 IU/ml of IL$_2$ (FIG. **13**D).

In conclusion, these results collectively show that LAG-3 and MHC Class II molecules, which are each T cellactivating antigens, may be likened to effector molecules involved in the phase of inactivation of T cell responses. Moreover, these results illustrate the importance of interactions between T cells in the control of the cellular immune response.

EXAMPLE 7

Stimulation of cell cytotoxicity by LAG-3Ig

The role of LAG-3Ig in relation to cell cytotoxicity is studied on two types of effector cells:

freshly drawn human peripheral blood lymphocytes (PBL),

S1B5 line cells (clone of human NK cells).

The cytotoxic activity of these cells is measured by counting the ^{51}Cr released into the medium by previously labelled target cells, in the presence or absence of LAG-3Ig in the medium.

FIG. **14** shows the degree of cytotoxicity of S1B5 for a line of human B cells transformed by Epstein-Barr virus and carrying major histocompatibility complex Class I and II antigens (LAZ 388 line), as a function of different reactants added to the cultures.

Measurements are carried out after 4 hours of coculture for effector/target (S1B5/LAZ 388) cell ratios of 3:1 (clear columns) or 1:1 (shaded columns).

The negative controls consist of medium alone (MED), the immunoadhesin CD8Ig and the monoclonal antibody 17.B4 (anti-LAG-3).

The positive controls consist of three different monoclonal antibodies:

antibody L243 directed against Class II DR antigens,

antibody 9.49 directed against Class II DR, DP, DQ antigens,

antibody W632 directed against human major histocompatibility complex Class I antigens.

Anti-HLA Class I (W632) or Class II (L243) antibodies increase the lysis of the target cells (and not the 17B4 control). The immunoadhesin LAG-3Ig increases the lysis. The CD8Ig control has no effect.

FIG. **15** shows the results of an experiment similar to the above, in which the cytotoxicity of PBL with respect to Daudi cells (HLA Class I$^-$) is measured, for effector/target ratios of 50:1 (clear columns) and 15:1 (shaded columns). The reactants added to the medium are the same as the ones used in the first experiment, except for antibody 9.49 and antibody 17.B4. Antibody 10H3 is an isotype IgG1 immunoglobulin specific for the CD45 surface antigen. It is used as negative control.

No change is observed with an antibody directed against major histocompatibility complex Class I antigens (W632).

The data from these two series of measurements shown that, compared to negative controls, LAG-3Ig activates the cytotoxicity of NK cells. This effect is similar to the one observed with antibodies directed against MHC Class II molecules.

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TABLE NO. 1

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
N	25.172370911	27.259836197	67.855064392	AP-n	40	n3	−0.5000	1
NN2	25.667764664	26.471963882	67.420585632	AP-n	40	hn	0.1300	2
CA	24.625223160	26.867494583	69.180244446	AP-n	40	ca	0.1200	3
NN1	24.393711090	27.474891663	67.220008850	AP-n	42	hn	0.1300	4
MA	23.936895370	27.680395126	69.464080811	AP-n	40	h	0.0700	5
C	25.662780726	26.773513794	70.350120544	AP-n	40	c'	0.3800	6
O	25.295415878	27.090747833	71.482070923	AP-n	40	o'	−0.4100	7
CB	23.766021729	25.587018967	68.990669250	AP-n	40	c2	−0.2600	8
MB1	23.060285568	25.72443695	68.152351379	AP-n	40	h	0.0700	9
MB2	24.413969040	24.744903564	68.686981201	AP-n	40	h	0.0700	10
CG	22.921775818	25.153419495	70.196960449	AP-n	40	c−	0.3400	11
DD1	22.069602966	25.929233551	70.676017761	AP-n	40	o−	−0.5700	12
DD2	23.115716934	24.009321213	70.667663574	AP-n	40	o−	−0.5700	13
M	26.906179428	26.304588318	70.124969482	SER	41	n	−0.5000	14
CA	27.860145569	25.912786484	71.207519531	SER	41	ca	0.1200	15
HN	27.120641708	26.221813202	69.126319885	SER	41	hn	0.2800	16
MA	27.374551773	25.088045120	71.766326904	SER	41	h	0.1000	17
C	28.252065659	27.005065918	72.271789551	SER	41	c'	−0.3800	18
O	27.987834930	28.200620651	72.115295410	SER	41	o'	−0.3800	19
CB	29.083601227	25.298025131	70.494880676	SER	41	c2	−0.1700	20
MB1	28.786190033	24.599395752	69.690521240	SER	41	h	0.1000	21
MB2	29.691858292	26.092912674	70.004081726	SER	41	h	0.1000	22
OG	29.905221939	24.578149796	71.424118042	SER	41	oh	−0.3800	23
HG	30.662555695	24.231645584	70.939277649	SER	41	ho	0.3500	24
N	28.857526779	26.558052063	73.387054443	GLY	42	n	−0.5000	25
CA	29.199718475	27.440891266	74.535232544	GLY	42	cg	0.0200	26
MN	29.251720428	25.616510391	73.267890930	GLY	42	hn	0.2800	27

TABLE NO. 1-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
MA1	28.520187378	28.312047958	74.591857910	GLY	42	h	0.1000	28
MA2	28.983697891	26.890144348	75.468444824	GLY	42	h	0.1000	29
C	30.691051483	27.875793457	74.601707458	GLY	42	c'	0.3800	30
O	31.504199982	27.026502609	74.980445862	GLY	42	o'	−0.3800	31
N	31.113182068	29.132183075	74.266487122	PRO	43	n	−0.4200	32
CA	32.858349609	29.476200104	74.126792908	PRO	43	ca	0.0600	33
HA	33.096603394	28.605407715	73.708091736	PRO	43	h	0.1000	34
CD	30.24075089	30.174203873	73.722633362	PRO	43	c2	0.0600	35
MD1	29.467987061	30.516298294	74.466743469	PRO	43	h	0.1000	36
MD2	29.664882660	29.799777985	72.838768005	PRO	43	h	0.1000	37
C	33.318023682	29.988374710	75.414916992	PRO	43	c'	0.3800	38
O	32.682361603	30.557033539	76.312332153	PRO	43	o'	−0.3800	39
CB	32.483139038	30.574260712	73.043136597	PRO	43	c2	−0.2000	40
MB1	33.350620270	31.263189316	73.049049377	PRO	43	h	0.1000	41
MB2	32.463790894	30.110534668	72.036743164	PRO	43	h	0.1000	42
CG	31.160949707	31.299722672	73.307487488	PRO	43	c2	−0.2000	43
MG1	31.279897690	32.024162292	74.137779236	PRO	43	h	0.1000	44
MG2	30.794561386	31.862808228	72.428352356	PRO	43	h	0.1000	45
K	34.683673859	29.902477264	75.503486633	PRO	44	n	−0.4200	46
CA	35.485736847	30.679145813	76.490524292	PRO	44	ca	0.0600	47
KA	35.018527985	30.645456314	77.491592407	PRO	44	h	0.1000	48
CD	35.509281158	29.014368057	74.655700684	PRO	44	c2	0.0600	49
MD1	35.411357880	29.247959137	73.577461243	PRO	44	h	0.1000	50
MD2	35.214973450	27.955932617	74.801994324	PRO	44	h	0.1000	51
C	35.700843811	32.172924042	76.063224792	PRO	44	c'	0.3800	52
O	36.448230743	32.477428436	75.126922607	PRO	44	o'	−0.3800	53
CB	36.779544830	29.842718124	76.547348022	PRO	44	c2	−0.2000	54
HB1	37.662769315	30.430650711	75.863670149	PRO	44	h	0.1000	55
HB2	36.667564392	29.027103424	77.288825989	PRO	44	h	0.1000	56
CG	36.940769196	29.250995636	75.143180867	PRO	44	c2	−0.2000	57
MG1	37.446662903	29.982709885	74.483322144	PRO	44	h	0.1000	58
MG2	37.553295135	26.329860667	75.134750366	PRO	44	h	0.1000	59
N	35.026676178	33.104183197	76.753837585	ALA	45	h	−0.5000	60
CA	35.034278870	34.544979095	76.400695801	ALA	45	ca	0.1200	61
MN	34.452354431	32.747509003	77.536170959	ALA	45	hn	0.2800	62
MA	35.105010986	34.659946442	75.298950195	ALA	45	h	0.1000	63
C	36.209384918	35.322113037	77.076705833	ALA	45	c'	0.3800	64
O	36.163528442	35.637268066	78.268325806	ALA	45	o'	−0.3800	65
CB	33.646369934	35.083190918	76.800727844	ALA	45	c3	−0.3000	66
MB1	33.534698486	36.150661469	76.535202026	ALA	45	h	0.1000	67
MB2	32.828392029	34.539138794	76.290328979	ALA	45	h	0.1000	68
MB3	33.465579987	35.001335144	77.890525818	ALA	45	h	0.1000	69
N	37.266757965	35.613758087	76.297294617	ALA	46	n	−0.5000	70
MN	37.216701508	35.231555939	75.346885681	ALA	46	hn	0.2800	71
CA	38.489383698	36.310386658	76.786270142	ALA	46	ca	0.1200	72
MA	38.262126923	36.871456146	77.716934204	ALA	46	h	0.1000	73
C	39.058414459	37.311935425	75.727844238	ALA	46	c'	0.3800	74
O	38.922710419	37.100418091	74.516731262	ALA	46	o'	−0.3800	75
CB	39.526046753	35.215301514	77.108406067	ALA	46	c3	−0.3000	76
MB1	40.446556091	35.633434296	77.555480957	ALA	46	h	0.1000	77
MB2	39.131206512	34.469978333	77.822120667	ALA	46	h	0.1000	78
MB3	39.821903229	34.663463593	76.197715759	ALA	46	h	0.1000	79
N	39.737388611	38.384365082	76.180320740	ALA	47	n	−0.5000	80
CA	40.295833588	39.429889679	75.275863647	ALA	47	ca	0.1200	81
MN	39.717037201	38.512592316	77.196357727	ALA	47	hn	0.2800	82
MA	39.901103973	39.219335938	74.245994568	ALA	47	h	0.1000	83
C	41.869789124	39.413467407	75.170806885	ALA	47	c'	0.3800	84
O	42.518333435	40.166862488	75.906578044	ALA	47	o'	−0.3800	85
CB	39.722030640	40.769996643	75.786285400	ALA	47	c3	−0.3000	86
MB1	40.045078278	41.611721039	75.145561218	ALA	47	h	0.1000	87
MB2	38.615306854	40.778987885	75.787467957	ALA	47	h	0.1000	88
MB3	40.059757233	41.007873535	76.813346863	ALA	47	h	0.1000	89
N	42.537422180	38.621597290	74.274406433	PRO	48	n	−0.4200	90
CA	44.009857178	38.718185425	74.045745850	PRO	48	ca	0.0600	91
MA	44.539390564	38.855972290	75.008773804	PRO	48	h	0.1000	92
CD	41.903011322	37.522361755	73.516281128	PRO	48	c2	0.0600	93
MD1	41.081176758	37.871139526	72.860626221	PRO	48	h	0.1000	94
MD2	41.490711312	36.761222839	74.206848145	PRO	48	h	0.1000	95
C	44.448683322	39.844142914	73.044319153	PRO	48	c'	0.3800	96
O	43.693031311	40.225761414	72.144134521	PRO	48	o'	−0.3800	97
CB	44.302902771	37.304885864	73.500595093	PRO	48	c2	−0.2000	98
MB1	45.227554321	37.245840894	72.897834778	PRO	48	h	0.1000	99
MB2	44.442562103	36.597515106	74.341529846	PRO	48	h	0.1000	100
CG	43.051033020	36.925685883	72.702011108	PRO	48	c2	−0.2000	101
MG1	43.084365845	37.389282227	71.696128845	PRO	48	h	0.1000	102

TABLE NO. 1-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
MG2	42.948661804	35.835418701	72.555740356	PRO	48	h	0.1000	103
N	45.700798035	40.324272156	73.165817261	GLY	49	n	−0.5000	104
CA	46.289730072	41.291931152	72.184875488	GLY	49	cg	0.0200	105
MN	46.207077026	39.986907959	73.991729736	GLY	49	hn	0.2800	106
MA1	45.620616913	41.460151672	71.317451477	GLY	49	h	0.1000	107
MA2	46.357063293	42.283206940	72.672386169	GLY	49	h	0.1000	108
C	47.682319641	40.950855255	71.600997925	GLY	49	c'	0.3800	109
O	48.560806274	41.811416626	71.601501465	GLY	49	o'	−0.3800	110
N	47.842975616	39.718383789	71.091018677	HIS	50	n	−0.5000	111
MN	46.947166443	39.222202301	71.052452087	HIS	50	hn	0.2800	112
CA	49.020114899	39.216835022	70.306198120	HIS	50	ca	0.1200	113
MA	49.324539185	38.296181652	70.836235046	HIS	50	h	0.1000	114
C	50.400863647	39.993576050	70.182159941	HIS	50	c'	0.3800	115
O	50.733478546	40.446022034	69.081466675	HIS	50	o'	−0.3800	116
CB	48.455345154	38.673969269	68.953773499	HIS	50	c2	−0.2000	117
HB1	49.238502502	38.051708221	68.481765747	HIS	50	h	0.1000	118
HB2	47.639427185	37.951225281	69.144165039	HIS	50	h	0.1000	119
CG	47.954322815	39.695293427	67.919425964	HIS	50	c5	0.1000	120
MD1	46.759342194	40.404747009	68.035293579	HIS	50	np	−0.4200	121
CE1	46.825572968	41.133701324	66.873245239	HIS	50	c5	0.2700	122
NE2	47.887611389	40.964004517	66.014495850	HIS	50	np	−0.5000	123
CD2	48.617565155	40.019775391	66.717041016	HIS	50	c5	0.0100	124
ME1	46.043247223	41.852840424	66.655242920	HIS	50	h	0.1300	125
ME2	48.092224121	41.403381348	65.108955383	HIS	50	hn	0.2800	126
MD2	49.566501617	39.599807739	66.396347046	HIS	50	h	0.1300	127
N	51.278770447	40.105480194	71.226615906	PRO	51	n	−0.4200	128
CA	52.667034149	40.618915558	71.077911377	PRO	51	ca	0.0600	129
MA	52.723186493	41.393623352	70.287094116	PRO	51	h	0.1000	130
CD	50.956447601	39.767082214	72.624496469	PRO	51	c2	0.0600	131
MD1	50.920970917	38.670558929	72.747383118	PRO	51	h	0.1000	132
MD2	49.988422394	40.190521240	72.947166443	PRO	51	h	0.1000	133
C	53.707103729	39.478122711	70.804489136	PRO	51	c'	0.3800	134
O	53.623699188	38.391666412	71.391418457	PRO	51	o'	−0.3800	135
CB	52.846694946	41.283794403	72.455955505	PRO	51	c2	−0.2000	136
MB1	53.907955170	41.442169189	72.729286194	PRO	51	h	0.1000	137
MB2	52.373264313	42.285846710	72.449127197	PRO	51	h	0.1000	138
CG	52.105823517	40.370025635	73.440398170	PRO	51	c2	−0.2000	139
MG1	52.782051086	39.565395355	73.789718628	PRO	51	h	0.1000	140
MG2	51.753723145	40.912036896	74.337333679	PRO	51	h	0.1000	141
N	54.704883575	39.729522705	69.933471680	LEU	52	n	−0.5000	142
CA	55.791530609	35.746330261	69.603820801	LEU	52	ca	0.1200	143
MN	54.653743744	40.652229308	69.490356445	LEU	52	hn	0.2800	144
MA	56.479202271	39.288208008	68.927917480	LEU	52	h	0.1000	145
C	35.301837921	37.525024414	68.745040894	LEU	52	c'	0.3800	146
O	55.637695313	37.425930023	67.562049866	LEU	52	o'	−0.3800	147
CB	56.671585083	38.316761017	70.829437256	LEU	52	c2	−0.2000	148
MB1	56.036743164	37.710464478	71.502593994	LEU	52	h	0.1000	149
MB2	57.445541382	37.602729797	70.487434387	LEU	52	h	0.1000	150
CG	57.363307953	39.420749664	71.675102234	LEU	52	c1	−0.1000	151
MG	56.617557526	40.200679779	71.926834106	LEU	52	h	0.1000	152
CD1	57.875057220	38.833915710	73.004547119	LEU	52	c3	−0.3000	153
MD11	58.353130341	39.601875305	73.642135620	LEU	52	h	0.1000	154
MD12	57.048751831	38.403957367	73.601577759	LEU	52	h	0.1000	155
MD13	58.618564606	38.028480530	72.852462769	LEU	52	h	0.1000	156
CD2	58.531608582	40.085853577	70.927200317	LEU	52	c3	−0.3000	157
MD21	59.028976440	40.857166290	71.545303345	LEU	52	h	0.1000	158
MD22	59.309509277	39.355545044	70.634819031	LEU	52	h	0.1000	159
MD23	58.192760468	40.592193604	70.005569458	LEU	52	h	0.1000	160
N	54.534648895	36.601863861	69.354270935	ALA	53	h	−0.5000	161
CA	53.940563202	35.420303345	68.673507690	ALA	53	ca	0.1200	162
MN	54.159572601	36.958141327	70.246543884	ALA	53	hn	0.2800	163
HA	53.600482941	35.753322601	67.671340942	ALA	53	h	0.1000	164
C	52.639778137	34.883575439	69.383995056	ALA	53	c'	0.3800	165
O	51.628326416	34.818698883	68.677047729	ALA	53	o'	−0.3800	166
CB	55.008785248	34.330768585	68.423698425	ALA	53	c3	−0.3000	167
MB1	54.582756042	33.460170746	67.892036438	ALA	53	h	0.1000	168
MB2	55.828662872	34.711517334	67.787284851	ALA	53	h	0.1000	169
MB3	55.479701896	33.961406708	69.351325989	ALA	53	h	0.1000	170
N	52.555103302	34.484893799	70.698265076	PRO	54	n	−0.4200	171
CA	51.304950714	33.917499542	71.286506653	PRO	54	ca	0.0600	172
MA	50.878768921	33.172523499	71.584587097	PRO	54	h	0.1000	173
CD	53.705833435	34.435420990	71.626182556	PRO	54	c2	0.0600	174
MD1	54.215991974	35.409980774	71.739936829	PRO	54	h	0.1000	175
MD2	54.453365326	33.693523407	71.288909912	PRO	54	h	0.1000	176
C	50.177021027	34.945980072	71.642837524	PRO	54	c'	0.3800	177

TABLE NO. 1-continued

Atom name	x	y	z	residue name and no.	atom type, charge and no.
O	50.377983093	36.164409637	71.677795410	PRO 54	o' −0.3800 178
CB	51.867893219	33.173828125	72.519187927	PRO 54	c2 −0.2000 179
MB1	51.135505676	33.051830292	73.340660095	PRO 54	h 0.1000 180
MB2	52.181377411	32.150829315	72.232276917	PRO 54	h 0.1000 181
CG	53.087123871	33.989192963	72.949317932	PRO 54	c2 −0.2000 182
MG1	52.768703461	34.871643066	73.538475037	PRO 54	h 0.1000 183
MG2	53.791828156	33.414070129	73.579086304	PRO 54	h 0.1000 184
N	48.977436066	34.414421082	71.936706543	GLY 55	n 0.5000 185
CA	47.822620392	35.225021362	72.404747009	GLY 55	cg 0.0200 186
MN	48.958133698	33.389709473	71.929512024	GLY 55	hn 0.2800 187
MA1	47.830284119	36.238574982	71.983676453	GLY 55	h 0.1000 188
MA2	46.896526337	34.772380829	72.004432678	GLY 55	h 0.1000 189
C	47.670829773	35.263648987	73.950416565	GLY 55	c' 0.3800 190
O	47.247509003	34.242198944	74.498336792	GLY 55	o' −0.3800 191
N	47.956153870	36.372848511	74.696281433	PRO 56	n −0.4200 192
CA	47.830066681	36.396587372	76.179130554	PRO 56	ca 0.0600 193
MA	48.225147247	35.457695007	76.619560242	PRO 56	h 0.1000 194
CD	48.653686523	37.556163788	74.163940430	PRO 56	c2 0.0600 195
MD1	48.108860016	38.040233612	73.332565308	PRO 56	h 0.1000 196
MD2	49.652721405	37.256084442	73.799308777	PRO 56	h 0.1000 197
C	46.361907959	36.804877472	76.668052673	PRO 56	c' 0.3800 198
O	45.890090942	37.732730865	76.845039368	PRO 56	o' −0.3800 199
CB	48.804897308	37.531764984	76.560951233	PRO 56	c2 −0.2000 200
MB1	48.542221069	38.034294128	77.511970520	PRO 56	h 0.1000 201
MB2	49.825592041	37.124942236	76.697402954	PRO 56	h 0.1000 202
CG	48.782566071	38.488700867	75.368530273	PRO 56	c2 −0.2000 203
MG1	47.903289795	39.158111572	75.434867859	PRO 56	h 0.1000 204
MG2	49.679012299	39.133480072	75.321792603	PRO 56	h 0.1000 205
N	45.650573730	35.488880157	76.896896362	HIS 57	n −0.5000 206
MN	46.046119690	34.657379150	76.439048767	HIS 57	hn 0.2800 207
CA	44.244419098	35.504474640	77.387596130	HIS 57	ca 0.1200 208
MA	43.667560577	36.207649231	76.759368896	HIS 57	h 0.1000 209
C	44.173530579	35.943927765	78.901000977	HIS 57	c' 0.3800 210
O	44.750030518	35.234142303	79.736030579	HIS 57	o' −0.3800 211
CB	43.610416412	34.095542908	77.185058594	HIS 57	c2 −0.2000 212
MB1	44.270858765	33.323795319	77.623748779	HIS 57	h 0.1000 213
MB2	42.689029694	34.034877777	77.796188354	HIS 57	h 0.1000 214
CB	43.250400543	33.671833038	75.751731873	HIS 57	c5 0.1000 215
MD1	44.116428375	33.723646257	74.666069031	HIS 57	np −0.4200 216
CE1	43.325973511	33.139041901	73.713264465	HIS 57	c5 0.2700 217
ME2	42.066158295	32.716590881	74.036811829	HIS 57	np −0.5000 218
CD2	42.045005798	33.048488617	75.379264832	HIS 57	c5 0.0100 219
ME1	43.722070465	33.001682281	72.711807251	HIS 57	h 0.1300 220
ME2	41.370864868	32.229383759	73.464263916	HIS 57	hn 0.3800 221
MD2	41.236827850	32.815635681	76.058380127	HIS 57	h 0.1300 222
N	43.513858795	37.068405151	79.318038940	PRO 58	n −0.4200 223
CA	43.593978882	37.569736481	80.719970703	PRO 58	ca 0.0600 224
MA	44.657642365	37.630668640	81.026939392	PRO 58	h 0.1000 225
CD	42.833599091	38.010932922	78.407653809	PRO 58	c2 0.0600 226
MD1	42.093353271	37.516807556	77.751007080	PRO 58	h 0.1000 227
MD2	43.569110870	38.527889252	77.758674622	PRO 58	h 0.1000 228
C	42.805988312	36.695770264	81.747009277	PRO 58	c' 0.3800 229
O	41.569808960	36.693984985	81.769706726	PRO 58	o' −0.3800 230
CB	43.072513580	39.016571045	80.579101563	PRO 58	c2 −0.2000 231
MB1	42.559799194	39.392253876	81.485702515	PRO 58	h 0.1000 232
MB2	43.920654297	39.706352234	80.398040771	PRO 58	h 0.1000 233
CG	42.156440735	38.999496460	79.353399631	PRO 58	c2 −0.2000 234
MG1	41.151851654	38.629653931	79.634307861	PRO 58	h 0.1000 235
MG2	42.023620605	39.998752594	78.896965027	PRO 58	h 0.1000 236
N	43.540618896	35.961261749	82.605430603	ALA 59	n −0.5000 237
MN	44.537330627	35.932937622	82.365699768	ALA 59	hn 0.2800 238
CA	42.949653625	34.946208954	83.526855469	ALA 59	ca 0.1200 239
MA	42.197692871	34.355514526	82.965911865	ALA 59	h 0.1000 240
C	42.208984375	35.488883972	84.803985596	ALA 59	c' 0.3800 241
O	42.496433258	35.127353668	85.948333740	ALA 59	o' −0.3800 242
CB	44.107444763	33.975612640	83.839767456	ALA 59	c3 −0.3000 243
MB1	43.761249542	33.130538940	84.463287354	ALA 59	h 0.1000 244
MB2	44.544620514	33.531585693	82.924507141	ALA 59	h 0.1000 245
MB3	44.925910950	34.467308044	84.399597168	ALA 59	h 0.1000 246
N	41.192062378	36.323741913	84.559051514	ALA 60	n −0.5000 247
MN	41.124549866	36.554992676	83.555343628	ALA 60	hn 0.2800 248
CA	40.203086853	36.790748596	85.562988281	ALA 60	ca 0.1200 249
MA	40.023948669	35.965785980	86.283721924	ALA 60	h 0.1000 250
C	38.823528290	37.027305602	84.846977234	ALA 60	c' 0.3800 251
O	37.897804260	36.283885956	85.186340332	ALA 60	o' −0.3800 252

TABLE NO. 1-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
CB	40.756233215	37.971691132	86.389617920	ALA	60	c3	−0.3000	253
MB1	40.004146578	38.357006073	87.102043152	ALA	60	h	0.1000	254
MB2	41.631908417	37.656120300	86.987319946	ALA	60	h	0.1000	255
MB3	41.090072632	38.818138123	85.765472412	ALA	60	h	0.1000	256
N	38.616340637	37.921970367	83.823921204	PRO	61	n	−0.4200	257
CA	37.403762817	37.874496460	82.948486328	PRO	61	ca	0.0600	258
MA	36.491020223	37.824863434	83.573593140	PRO	61	h	0.1000	259
CD	39.556003571	39.003845215	83.459777832	PRO	61	c2	0.0600	260
MD1	40.594814301	38.658158052	83.304046631	PRO	61	h	0.1000	261
MD2	39.571029663	39.778694153	84.250648498	PRO	61	h	0.1000	262
C	37.275394440	36.712963104	81.892074585	PRO	61	c'	0.3800	263
O	36.366387939	36.670307159	81.183484568	PRO	61	o'	−0.3800	264
CB	37.473857880	39.266963959	82.286987305	PRO	61	c2	−0.2000	265
MB1	36.949714661	39.321308136	81.312759399	PRO	61	h	0.1000	266
MB2	36.985511780	40.017913818	82.938987732	PRO	61	h	0.1000	267
CG	38.968631744	39.571613312	82.168632507	PRO	61	c2	−0.2000	268
HG1	39.397396088	39.046298981	81.292793274	PRO	61	h	0.1000	269
HG2	39.180202484	40.649600983	82.039207458	PRO	61	h	0.1000	270
N	38.242324829	35.777751923	81.785034180	SER	62	n	−0.5000	271
CA	38.186004639	34.624855042	80.844123840	SER	62	ca	0.1200	272
MN	39.035541534	35.827360535	82.416992185	SER	62	hn	0.2800	273
MA	37.921787262	35.006183624	79.841125488	SER	62	h	0.1000	274
C	37.145660400	33.532897949	81.261909485	SER	62	c'	0.3800	275
O	37.466091156	32.626064301	82.041137695	SER	62	o'	−0.3800	276
CB	39.620265961	34.048240662	80.725196835	SER	62	c2	−0.1700	277
MB1	39.660293579	33.306644440	79.904281616	SER	62	h	0.1000	278
MB2	40.349685669	34.832687378	80.442802429	SER	62	h	0.1000	279
OG	40.032703400	33.414188385	81.938880920	SER	62	oh	−0.3800	280
MG	39.252223969	32.931293488	82.256263733	SER	62	ho	0.3500	281
N	35.902244568	33.647918701	80.764541626	SER	63	n	−0.5000	282
CA	34.747528076	32.874965942	81.297317505	SER	63	cg	0.1200	283
MN	35.768447876	34.503852844	80.205200195	SER	63	hn	0.2800	284
MA	35.064254761	32.265518188	82.170570374	SER	63	h	0.1000	285
C	34.106758118	31.936998367	80.231674194	SER	63	c'	0.3800	286
O	33.716896657	32.367130280	79.142120361	SER	63	o'	−0.3800	287
CB	33.716815948	33.889484406	81.843544006	SER	63	c2	−0.1700	288
MB1	34.199871063	34.571354430	82.572105408	SER	63	h	0.1000	289
MB2	33.22880719	34.543502808	81.036796570	SER	63	h	0.1000	290
OG	33.634590149	33.222091675	82.496467590	SER	63	oh	−0.3800	291
MG	32.159793854	32.710407257	81.832328796	SER	63	ho	0.3500	292
N	33.914913177	30.658897400	80.588378906	TRP	64	n	−0.5000	293
CA	33.112319946	29.694124222	79.783546448	TRP	64	ca	0.1200	294
MN	34.221500397	30.422899246	81.538955658	TRP	64	hn	0.2800	295
MA	33.404731750	29.812168121	78.721931458	TRP	64	h	0.1000	296
C	31.573041916	29.961977005	79.883049011	TRP	64	c'	0.3800	297
O	30.996980667	29.940891266	80.975959778	TRP	64	o'	−0.3800	298
CB	33.525466919	26.235471725	80.142707825	TRP	64	c2	−0.2000	299
MB1	32.950366974	27.534402847	79.508041382	TRP	64	h	0.1000	300
MB2	34.571674347	28.078489304	79.816658020	TRP	64	h	0.1000	301
CG	33.405326843	27.783784866	81.611763000	TRP	64	c5	0.0000	302
CD1	32.267101288	27.214570999	82.221214294	TRP	64	c5	0.1000	303
NE1	32.481933594	26.953943253	83.590408325	TRP	64	np	−0.5000	304
CE2	33.781952422	27.378627777	83.812339783	TRP	64	c5	0.1100	305
CD2	34.355152230	27.881061554	82.617965698	TRP	64	c5	0.0000	306
MD1	31.322025528	27.036033630	81.708480835	TRP	64	h	0.1000	307
ME1	31.820940018	26.578481674	84.279945374	TRP	64	hn	0.2800	308
CE3	35.681034088	28.394184113	82.615905762	TRP	64	cp	−0.1000	309
ME3	36.128986359	28.784986496	81.714393616	TRP	64	h	0.1000	310
CE3	36.396430969	28.387191772	83.815704346	TRP	64	cp	−0.1000	311
ME3	37.405311584	28.776082993	83.832160950	TRP	64	h	−0.1000	312
CM2	35.830604553	27.888952255	84.994210925	TRP	64	cp	0.1000	313
MM2	36.410472870	27.896844844	85.506951904	TRP	64	h	0.1000	314
CZ2	34.527233124	27.382776260	85.014289856	TRP	64	cp	0.1000	315
MZ2	34.097515106	27.006088257	85.929389954	TRP	64	h	0.1000	316
N	30.921349498	30.232547760	78.740600586	GLY	65	n	0.5000	317
CA	29.460748672	30.504768372	78.692192078	GLY	65	cg	0.0200	318
MN	31.520374298	30.302478790	77.901519775	GLY	65	hn	0.2800	319
MA1	29.073087692	30.896234512	79.650825500	GLY	65	h	0.1000	320
MA2	29.288171768	31.333106995	77.981094360	GLY	65	h	0.1000	321
C	28.633579254	29.293350220	78.197364807	GLY	65	c'	0.3800	322
O	25.866907883	29.137302399	76.975486755	GLY	65	o'	−0.3800	323
N	27.969013672	28.429246902	79.038352966	PRO	66	n	0.0200	324
CA	27.282257080	27.212890625	78.846752930	PRO	66	ca	0.0600	325
MA	27.989650726	26.634012222	77.917152405	PRO	66	h	0.1000	326
CD	28.016592026	28.832529831	80.511337280	PRO	66	c2	0.0600	327

TABLE NO. 1-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
MD1	27.731332779	29.836006927	80.880874634	PRO	66	h	0.1000	328
MD2	29.027824402	28.301166534	80.897590637	PRO	66	b	0.1000	329
C	25.977466583	27.479314804	77.725341797	PRO	66	c'	0.3800	330
O	25.217950821	25.417282104	77.982574463	PRO	66	o'	−0.3800	331
CB	27.045602798	26.422634125	79.851196258	PRO	66	c2	−0.2000	332
MB1	26.132562637	25.797079086	79.827728271	PRO	66	h	0.1000	333
MB2	27.890687943	25.729280472	80.029365540	PRO	66	h	−0.1000	334
CG	27.003501892	27.477243423	80.958015442	PRO	66	c2	−0.2000	335
MG1	25.990892410	27.921483994	81.014678955	PRO	66	h	0.1000	336
MH2	27.232566833	27.061700821	81.956459045	PRO	66	h	0.1000	337
N	25.734319687	26.626403809	76.719802856	ARG+	67	n	−0.5000	335
CA	24.603988647	26.793025970	75.767257690	ARG+	67	ca	0.1200	339
MN	26.386735916	25.841371536	76.649803162	ARG+	67	hn	0.2800	340
MA	24.496238708	27.874828339	75.561668396	ARG+	67	h	0.1000	341
C	23.227464676	26.224872589	76.267372131	ARG+	67	c'	0.2800	342
O	23.178310394	25.034952164	76.603759766	ARG+	67	o'	−0.3800	343
CB	24.990055899	26.165229797	74.398826599	ARG+	67	c2	−0.2000	344
MB1	24.135663986	26.318639755	73.709175110	ARG+	67	h	0.1100	345
MB2	25.787433624	26.779323578	73.940032959	ARG+	67	h	0.1100	346
CG	25.439929962	24.676465988	74.361564636	ARG+	67	c2	−0.2000	347
MG1	26.546255112	24.646316528	74.415458679	ARG+	67	h	0.1300	348
MG2	25.092346191	24.131168365	75.261718750	ARG+	67	h	0.1300	349
CD	24.934387207	23.941221237	73.112297058	ARG+	67	c2	−0.0900	350
MD	23.838283539	23.774566650	73.188652039	ARG+	67	h	0.1300	351
MD2	25.070211411	24.585262299	72.220893860	ARG+	67	h	0.1300	352
ME	25.665744781	22.657058716	72.968780518	ARG+	67	n1	−0.5000	353
ME	26.251846313	22.313375473	73.731925964	ARG+	67	hn	0.3600	354
CZ	25.689014435	21.902687073	71.871635437	ARG+	67	cr	0.4500	355
MM1	26.493299484	20.879484177	71.859733582	ARG+	67	n2	−0.5000	356
M11	27.072875705	20.740955353	72.690315247	ARG+	67	hn	0.3600	357
MM12	26.520929337	20.119580078	71.006805420	ARG+	67	hn	0.3600	358
MM2	24.956668854	22.117029190	70.805320740	ARG+	67	n2	−0.5000	359
MM21	25.030595779	21.456792831	70.033142090	ARG+	67	hn	0.3600	360
MM22	24.266489029	22.916227341	70.550784302	ARG+	67	hn	0.3600	361
N	22.080270767	26.971176147	76.244255066	ARG+	68	n	−0.4200	362
CA	20.743734360	24.358839035	76.485237122	PRO	68	ca	0.0600	363
MA	20.817556381	25.605155945	77.294357300	PRO	68	h	0.1000	364
CD	22.076143265	28.448001862	76.342918396	PRO	68	c2	0.0600	365
MD1	22.539228439	25.949869I56	75.469612122	PRO	68	h	0.1000	366
MD2	22.632146835	25.776126862	77.244499207	PRO	68	h	0.1000	367
C	20.182382584	25.586324692	75.240180969	PRO	68	c'	0.3800	368
O	20.420539856	24.381649017	75.139877319	PRO	68	o'	−0.3800	369
CB	19.948141098	27.549791336	77.062515259	PRO	68	c2	−0.2000	370
MB1	18.858430862	27.490163803	76.877128601	PRO	68	h	0.1000	371
MB2	20.066789627	27.567596436	78.163002014	PRO	68	h	0.1000	372
CG	20.592071533	28.804227829	76.667755179	PRO	68	c2	−0.2000	373
MG1	20.158363342	29.031393051	75.478172302	PRO	68	h	0.1000	374
MG2	20.428398132	29.704229355	77.092338562	PRO	68	h	0.1000	375
N	19.458055496	26.229068756	74.300010681	ARG+	69	n	−0.5000	376
CA	18.893756866	25.542047501	73.096710205	ARG+	69	ca	0.1200	377
MN	19.221296310	27.198928833	74.529014587	ARG+	69	hn	0.2800	378
MA	19.667610168	24.872461319	72.660797119	ARG+	69	h	0.1000	379
C	18.514461517	26.608341217	72.012504375	ARG+	69	c'	0.3800	380
O	17.383218765	27.099323273	72.036094666	ARG+	69	o'	−0.3800	381
CB	17.681777954	24.654710770	73.539657593	ARG+	69	c2	−0.2000	352
MB1	18.011884689	23.935596466	74.315147400	ARG+	69	h	0.1100	383
MB2	16.954420090	25.310214996	74.061965942	ARG+	69	h	0.100	384
CG	16.946891785	23.862810135	72.427490234	ARG+	69	c2	−0.2000	385
MG1	16.649517059	24.851628113	71.612152100	ARG+	69	h	0.1300	386
MG2	17.638776779	23.127803802	71.968805054	ARG+	69	h	0.1300	387
CD	15.688191414	23.166488647	72.975959775	ARG+	69	c2	−0.9000	388
MD1	15.980383873	22.365550995	73.686569214	ARG+	69	h	0.1300	389
MD2	15.090404510	23.898859024	73.554351807	ARG+	69	h	0.1300	390
ME	14.889810562	22.612804413	71.848815915	ARG+	69	n1	−0.5000	391
ME	15.272388458	22.616128922	70.898582458	ARG+	69	hn	0.3600	392
CZ	13.644455910	22.143890381	71.942466736	ARG+	69	cr	0.4500	393
MM1	13.048615456	21.755460739	70.850708005	ARG+	69	n2	−0.5000	394
MM11	13.576630592	21.832328796	69.979103058	ARG+	69	hn	0.3600	395
MM12	12.090744019	21.411947250	70.936050933	ARG+	69	hn	0.3600	396
MM2	12.989639282	22.056533813	73.074552507	ARG+	69	n2	−0.5000	397
CZ	13.644455910	22.143590381	71.942466736	ARG+	69	cr	0.4500	393
MM1	13.048615456	21.755460739	70.850705008	ARG+	69	n2	−0.500	394
MM11	13.576630592	21.822325796	69.979103088	ARG+	69	hn	0.3600	395
MM12	12.090744019	21.411947250	70.936080933	ARG+	69	hn	0.3600	396
MM2	12.989639282	22.056533813	73.074882507	ARG+	69	n2	−0.5000	397

TABLE NO. 1-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
MM21	12.033639908	21.696094513	73.066261292	ARG+	69	hn	0.3600	398
MM22	13.529273987	22.372974396	73.883934021	ARG+	69	hn	0.3600	399
N	19.436628342	26.928932190	71.074501038	ARG+	70	n	−0.5000	400
CA	19.223009109	27.811206818	69.878326416	ARG+	70	ca	0.1200	401
MN	20.357131958	26.451332092	71.165985107	ARG+	70	hn	0.2800	402
MA	19.087514877	27.065124512	69.071128845	ARG+	70	h	0.1000	403
C	20.512538910	28.575149536	69.398536682	ARG+	70	c'	0.3800	404
O	20.872812271	25.468791962	68.228363037	ARG+	70	o'	−0.3800	405
CB	17.935552597	28.697717667	69.732887268	ARG+	70	c2	−0.2000	406
MB1	17.889257431	29.085472107	68.694030762	ARG+	70	h	0.1100	407
MB2	17.053388596	28.033058167	69.807891846	ARG+	70	h	0.1100	408
CG	17.775762558	29.883768082	70.717842102	ARG+	70	c2	−0.2000	409
MG1	18.004568100	29.946030045	71.747383118	ARG+	70	h	0.1300	410
MG2	18.540782928	30.651863098	70.495643616	ARG+	70	h	0.1300	411
CD	16.368116379	30.502414703	70.693214417	ARG+	70	c2	−0.0900	412
MD1	16.095691681	30.812221527	69.664886475	ARG+	70	h	0.1300	413
MD2	15.630161285	29.711160660	70.937812805	ARG+	70	h	0.1300	414
ME	16.255954742	31.590759277	71.711013794	ARG+	70	n1	−0.5000	415
ME	16.253637314	31.350564957	72.706428528	ARG+	70	hn	0.3600	416
CE	16.144437790	32.900745392	71.464561462	ARG+	70	cr	0.4500	417
MM1	16.055492401	33.712893625	72.481109619	ARG+	70	n2	−0.5000	418
MM11	16.071464539	33.294216156	73.413330078	ARG+	70	hn	0.3600	419
MM12	15.975571632	34.708621979	72.277374268	ARG+	70	hn	0.3600	420
MM2	16.120351791	33.420074463	70.259872437	ARG+	70	n2	−0.5000	421
MM21	16.025018692	34.432674408	70.167800903	ARG+	70	hn	0.3600	422
MM22	16.187112808	32.736862183	69.505996704	ARG+	70	lm	0.3600	423
N	21.115812302	29.562902451	70.071769714	TYRC	71	n	−0.5000	424
MN	21.515977859	30.157514572	69.338050842	TYRC	71	hn	0.2800	425
CA	22.034273148	29.314456940	71.218978882	TYRC	71	ca	0.1200	426
MA	22.671009064	28.444923401	70.976676941	TYRC	71	h	0.1000	427
C	21.352920532	25.953493118	72.563385010	TYRC	71	c'	0.4100	428
OCT	20.392858505	29.553161621	73.048652649	TYRC	71	o'	−0.3800	429
Q	21.928325653	27.853042603	73.145797729	TYRC	71	oh	−0.3800	430
MO	21.429273605	27.558662415	73.909782410	TYRC	71	ho	0.3500	431
CB	22.969152451	30.555358887	71.361984253	TYRC	71	c2	−0.2000	432
MB1	22.368267059	31.487627029	71.355415344	TYRC	71	h	0.1000	433
MB2	23.401992798	30.559690475	72.381835938	TYRC	71	h	0.1000	434
CG	24.144546509	30.666173935	70.352111816	TYRC	71	cp	0.0000	435
CD1	23.927537372	31.126276016	69.044418335	TYRC	71	cp	−0.1000	436
MD1	22.944635391	31.424587630	68.717597961	TYRC	71	h	0.1000	437
CE1	24.987041473	31.212265015	68.143028259	TYRC	71	cp	−0.1000	438
ME1	24.819047928	31.555503845	67.131973267	TYRC	71	h	0.1000	439
CZ	26.273118973	30.861675262	68.542274475	TYRC	71	cp	0.0300	440
OM	27.314481735	30.957365036	67.652267456	TYRC	71	oh	−0.3800	441
MN	26.980180740	31.236070633	66.796859741	TYRC	71	ho	0.3500	442
CE2	26.504697800	30.422637939	69.841377258	TYRC	71	cp	−0.1000	443
ME2	27.503232956	30.148866653	70.140815735	TYRC	71	h	0.1000	444
CD2	25.447391510	30.325349808	70.743820190	TYRC	71	cp	−0.1000	445
MD2	25.652494431	29.968608856	71.745338440	TYRC	71	h	0.1000	446

*The numbering of the amino acids is shifted by minus 6 relative to the sequence SEQ ID No. 1.

TABLE NO. 2

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
N	24.753730401	26.435615520	68.246300542	CYSn	40	nj	−0.500	1
CA	24.503000259	26.336292725	69.707687375	CYSn	40	ca	0.1200	2
NN1	23.561560822	26.429992676	67.734397888	CYSn	40	hn	0.1400	3
NN2	25.250690460	25.603437424	67.909477234	CYSn	40	hn	0.1400	4
NA	23.590571594	27.247760773	69.940755269	CYSn	40	h	0.100	5
C	25.747190475	26.505004883	70.632949529	CYSn	40	c'	0.3800	6
O	25.611124039	27.204542160	71.634971619	CYSn	40	o'	−0.3800	7
CB	23.602088928	25.125436783	69.979545593	CYSn	40	c2	−0.3000	8
HB1	22.555475225	25.378625570	69.716011047	CYSn	40	h	0.1000	9
HB2	23.565217209	24.277284622	69.317871094	CYSn	40	h	0.1000	10
SG	23.623842010	24.466741562	71.679084778	CYSn	40	s1	0.1000	11
N	26.910152435	25.8S1416321	70.350471497	SER	41	n	−0.5000	12
CA	28.052598953	25.721915930	71.310394287	SET	41	ca	0.1200	13
HN	26.922674725	25.473436356	69.412322998	SER	41	hn	0.2800	14

TABLE NO. 2-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
RA	27.761577606	24.886217117	71.978584290	SER	41	h	0.1000	15
C	28.477056503	26.929338455	72.226699529	SER	41	c'	0.3800	16
O	25.412267685	28.097608566	71.829902649	SER	41	o'	−0.3800	17
CB	28.257335662	25.212779999	70.480110168	SER	41	c2	−0.1700	18
KB1	28.957101822	24.401222229	69.786743164	SER	41	h	0.1000	19
KB2	29.657650511	26.030597657	69.845893860	SER	41	h	0.1000	20
CG	30.284814825	24.713315964	71.339111328	SER	41	ch	−0.3800	21
NG	31.027490616	24.448732376	70.755003662	SER	41	hc	0.3500	22
N	28.904022217	24.617259979	73.466476440	GLY	42	n	−0.5000	23
CA	29.226711273	27.639400482	74.497131348	GLY	42	cg	0.0200	24
HX	29.140472412	25.626163483	73.587532043	GLY	42	hn	0.2800	25
KA1	28.567264557	28.519987106	74.394309988	GLY	42	h	0.1000	26
KA2	28.949186325	27.229663849	75.483924866	GLY	42	h	0.1000	27
C	30.728670120	28.040773392	74.587509155	GLY	42	c'	0.3800	28
O	31.522300720	27.171119690	74.961143494	GLY	42	o'	−0.3800	29
N	31.175983429	29.296203613	74.282577515	PRO	43	n	−0.4200	30
CA	32.627410889	29.612504959	74.140579224	PRO	43	ca	0.0600	31
KA	33.153442353	28.711788635	73.723632813	PRO	43	h	0.1000	32
CO	30.295356750	30.374078751	73.784042358	PRO	43	c2	0.0600	33
KD1	29.605636597	30.735929489	74.571166992	PRO	43	h	0.1000	34
KD2	29.653467865	30.826647568	72.928161621	PRO	43	h	0.1000	35
C	33.389945984	30.112844467	75.429031372	PRO	43	c'	0.3800	36
O	32.754360199	30.681560516	76.327354431	PRO	43	o'	−0.3800	37
CB	32.565906525	30.710325241	73.057388306	PRO	43	c2	−0.2000	38
KB1	33.449081421	31.378034592	73.055488586	PRO	43	h	0.1000	39
KB2	31.524932861	30.247560501	72.051818848	PRO	43	h	0.1000	40
CG	31.263490677	31.466632843	73.332626343	PRO	43	c2	−0.2000	41
KG1	31.413110733	32.204200745	74.146759733	PRO	43	h	0.1000	42
KG2	30.894020081	32.022109985	72.450286865	PRO	43	h	0.1000	43
N	34.754848480	30.015562057	75.523460388	PRO	44	n	−0.4200	44
CA	35.553565979	30.763086319	76.536285400	PRO	44	ca	0.6000	45
KA	35.083564758	30.695350647	77.536567688	PRD	44	h	0.1000	46
CD	35.574893951	29.325835648	74.665214519	PRO	44	c2	0.0600	47
KD1	35.471595764	29.373666763	73.588935852	PRO	44	h	0.1000	48
KD2	35.281650543	28.076414108	74.807395935	PRO	44	h	0.1000	49
C	35.767509460	32.265411377	76.141123281	PRO	44	c'	0.3300	50
O	36.544441223	32.599441528	75.238464355	PRD	44	o'	0.3800	51
CB	36.549227905	29.927103043	76.567779541	PRO	44	c2	−0.2000	52
HB1	37.732776642	30.502979279	76.899909705	PRO	44	h	0.1000	53
HB2	36.733722657	29.095364598	72.253833069	PRO	44	h	0.1000	54
CG	37.005489349	29.369808197	75.152618408	PRO	44	c2	−0.2000	55
MG1	37.502185822	30.119005203	74.504158020	PRO	44	h	0.1000	56
MG2	37.625408173	28.451385498	75.115615845	PRO	44	h	0.1000	57
N	35.047088623	33.173435211	76.816978455	ALA	45	n	−0.5000	58
CA	35.021333466	34.699920502	76.449218750	ALA	45	ca	0.1200	59
MN	34.471403029	32.798248291	77.590728760	ALA	45	hn	0.2800	60
HA	35.065380096	34.699813843	75.343650818	ALA	45	h	0.1000	61
C	36.187728882	35.414947510	77.090148926	ALA	45	c'	0.3800	62
O	36.133388519	35.819305420	78.255142212	ALA	45	o'	−0.3800	63
CB	33.615478516	35.135440826	76.831176758	ALA	45	c3	−0.3000	64
HB1	33.490375519	36.157480927	76.517280579	ALA	45	h	0.1000	65
HB2	32.811222076	34.556259155	76.338432312	ALA	45	h	0.1000	66
HB3	33.433517456	35.098365784	77.922439575	ALA	45	h	0.1000	67
N	37.264499664	35.613568713	76.306388855	ALA	46	n	−0.5000	68
HN	37.248532703	35.072978973	75.433502197	ALA	46	hn	0.2800	69
CA	38.503662109	36.398694611	76.764076233	ALA	46	ca	0.1200	70
MA	38.303600311	36.883266449	77.688095093	ALA	46	h	0.1000	71
C	39.082061765	37.173509979	75.687866211	ALA	46	c'	0.3800	72
O	38.951850891	37.052509308	74.481193542	ALA	46	c'	0.3800	73
CB	39.509185791	35.179004669	77.103065491	ALA	46	c3	−0.3000	74
HB1	40.441535950	35.582756042	77.525072327	ALA	46	h	0.1000	75
HB2	39.106670380	34.460605622	77.839447021	ALA	46	h	0.1000	76
HB3	39.780502319	34.997728729	76.205062866	ALA	46	h	0.1000	77
N	39.768814087	38.344066620	76.133750916	ALA	47	n	0.5000	78
CA	40.322643280	39.391151428	75.225708008	ALA	47	ca	0.1200	79
MN	39.783836365	38.455593109	77.149337769	ALA	47	hn	0.2800	80
HA	39.932807922	39.265201569	74.196365356	ALA	47	h	0.1000	81
C	41.900882721	39.401574817	75.126977349	ALA	47	c'	0.3800	82
O	42.538444519	40.171451569	75.854652405	ALA	47	o'	−0.3800	83
CB	39.728843659	40.731719971	75.714279175	ALA	47	c3	−0.3000	84
HB1	40.043342590	41.567916570	75.059875488	ALA	47	h	0.1000	85
HB2	38.621978760	40.726497630	75.711242676	ALA	47	h	0.1000	86
HB3	40.062076569	40.987442017	76.739311218	ALA	47	h	0.1000	87
N	42.578651428	38.603999056	74.242019653	PRO	48	n	−0.4200	88
CA	44.052674976	38.702857971	74.013595581	PRO	48	ca	0.0600	89

TABLE NO. 2-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
HA	44.576034546	38.850185394	74.977058411	PRO	48	h	0.1020	90
CO	41.956359863	37.474979401	73.520225525	PRO	48	c2	0.0600	91
HD1	41.114963531	37.378272858	72.872795105	PRO	48	h	0.1000	92
HD2	41.576156616	36.723918915	74.239135742	PRO	48	h	0.1000	93
C	44.492458344	39.820354462	73.002609253	PRO	48	c'	0.3800	94
O	43.782276154	40.131282806	72.040626526	PRO	48	o'	−0.3800	95
CB	44.356296539	37.288743516	73.479736328	PRO	48	c2	−0.2000	96
HB1	45.273612976	37.234390259	72.865592957	PRO	48	h	0.1000	97
HB2	44.813816833	36.591526031	74.322021484	PRO	48	h	0.1000	98
CG	43.102409363	36.884414673	72.702988770	PRO	48	c2	−0.2000	99
HG1	43.119277954	37.331111908	71.685241699	PRO	48	h	0.1000	100
HG2	43.010280609	35.788948059	72.572326660	PRO	48	h	0.1000	101
N	45.709655762	40.366821289	73.185493469	GLY	49	n	−0.5000	102
CA	46.317604065	41.332912445	72.214889526	GLY	49	cg	0.0200	103
HN	46.169986725	40.089691162	74.058357239	GLY	49	hn	0.2800	104
HA1	45.654991150	41.537052153	71.351181020	GLY	49	h	0.1000	105
HA2	46.406318665	42.313266754	72.719123840	GLY	49	b	0.1000	106
C	47.710880280	40.963481903	71.854037476	GLY	49	c'	0.3800	107
O	48.630664825	41.772521973	71.754951477	GLY	49	o'	−0.3800	108
N	47.830738068	39.763301849	71.063682556	HIS	50	n	−0.5000	109
HN	46.918842316	39.310573578	70.943237305	HIS	50	hn	0.2800	110
CA	49.045799255	39.210880280	70.375061035	HIS	50	ca	0.1200	111
HA	49.315334220	38.320884705	70.972137451	HIS	50	h	0.1000	112
C	50.433021545	39.981941223	70.267852783	HIS	50	c'	0.3800	113
O	50.773132324	40.456230164	69.178672791	HIS	50	o'	−0.3800	114
CB	28.558776855	32.590164165	69.024101257	HIS	50	c2	−0.2000	115
HB1	49.390335083	32.009521484	68.577232361	HIS	50	h	0.1000	116
HB2	47.792594910	37.817192078	69.227330181	HIS	50	b	0.1000	117
CG	47.597627258	39.545143127	67.956726074	HIS	50	c5	0.1000	118
HD1	46.669281006	39.956676482	67.918121338	HIS	50	np	0.1000	119
CE1	46.730144501	40.789539337	66.829002380	HIS	50	c5	0.2700	120
NE2	47.911670685	40.950614929	66.152328491	HIS	50	np	−0.5000	121
CD2	48.729324341	40.126430511	66.304235840	HIS	50	c5	0.0100	122
HE1	45.843067169	41.327060699	66.517631531	HIS	50	h	0.1300	123
HE2	48.138290425	41.548683167	65.349815369	HIS	50	hn	0.2800	124
HB2	49.789726257	39.981491089	66.738136292	HIS	50	h	0.1300	125
H	51.307849884	40.071182251	71.317932129	PRO	51	n	−0.4200	126
CA	52.692558239	40.596851349	71.184913635	PRO	51	ca	0.0600	127
HA	52.742668152	41.390510559	70.412712097	PRO	51	h	0.1000	128
CD	50.980678558	39.703777313	72.706970215	PRO	51	c2	0.0600	129
HD1	50.998199463	38.605384827	72.818397522	PRO	51	h	0.1000	130
HD2	49.987606049	40.071315765	73.019950867	PRO	51	b	0.1000	131
C	53.739063263	39.471630096	70.880722046	PRO	51	c'	0.3800	132
O	53.708900452	38.294466400	71.488830566	PRO	51	c'	−0.2800	133
CB	52.868911743	41.240936279	72.572486877	PRO	51	c2	0.2000	134
HB1	53.929355621	41.264253998	72.864852905	PRO	51	b	0.1000	135
HB2	52.429229736	42.258647919	72.365872192	PRO	51	h	0.1000	136
CG	52.087848663	40.349472046	73.547019958	PRO	51	c2	−0.2000	137
HG1	51.750400543	39.566276550	73.963310242	PRO	51	h	0.1000	138
HG2	51.686916351	40.923263550	74.403717041	PRO	51	h	0.1000	139
H	54.676445007	39.726749420	69.946899414	LEU	52	n	−0.5000	140
CA	55.768096924	38.764469147	69.270259094	LEU	52	ca	0.1200	141
HN	54.573589325	40.637012482	69.488586426	LEU	52	hn	0.2800	142
HA	56.414031982	39.225927734	68.869346619	LEU	52	h	0.1000	143
C	55.281269073	37.240004730	68.718757629	LEU	52	c'	0.2800	144
O	55.654800415	37.411125183	67.550910950	LEU	52	o'	−0.2800	145
CB	56.713882446	38.254763031	70.751991272	LEU	52	c2	−0.2000	146
HB1	56.125553131	37.737205505	71.456863403	LEU	52	h	0.1000	147
HB2	57.488136292	37.658962250	70.274801636	LEU	52	h	0.1000	148
CG	57.411731720	39.487998962	71.552589417	LEU	52	c1	−0.1000	149
HG	56.652648926	40.244640350	71.834617615	LEU	52	h	0.1000	150
CD1	58.010108948	38.936943054	72.859535217	LEU	52	c3	0.2000	151
HD11	58.475826263	39.735752106	73.467353821	LEU	52	h	0.1000	152
HD12	57.236072540	38.469894409	73.497505188	LEU	52	h	0.1000	153
HD13	38.787303925	38.171112061	72.675750732	LEU	52	h	0.1000	154
CD2	58.537623901	40.187110901	70.742630205	LEU	52	c1	0.3000	155
HD21	58.993679047	41.001243591	71.321037292	LEU	52	b	0.1000	156
HD22	59.321178436	39.487018585	70.445312500	LEU	52	h	0.1000	157
HD23	58.125030518	40.647811890	69.818283082	LEU	52	b	0.1000	158
H	54.475013733	36.643035889	69.215246582	ALA	53	n	0.5000	159
CA	53.896503448	35.451416016	68.639259338	ALA	53	ca	0.1200	160
HN	54.100524902	37.007503510	70.205250713	ALA	53	hn	0.2800	161
HA	53.553531647	35.773445129	67.634338379	ALA	53	h	0.1000	162
C	52.602260590	34.908508301	69.253744507	ALA	53	c'	0.3800	163
O	51.589200881	34.828308105	68.650024414	ALA	53	o'	−0.3800	164

TABLE NO. 2-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
CB	54.970031738	34.364234924	68.394615173	ALA	53	c3	−0.3000	165
HB1	54.534633636	33.449993134	67.949513843	ALA	53	h	0.1000	166
HB2	55.742454529	34.720954895	67.688003540	ALA	53	h	0.1000	167
HB3	55.500236511	34.068145752	69.316299435	ALA	53	h	0.1000	165
H	52.528435568	34.519321442	70.670552661	PRO	54	h	−0.4200	169
CA	51.258402557	33.944232941	71.268875122	PRO	54	ca	0.0600	170
HA	50.560397339	33.194515228	70.573081970	PRO	54	h	0.1000	171
CD	53.678298950	34.513732910	71.601514270	PRO	54	c2	0.0600	172
HD1	54.146690369	35.509193420	71.717323303	PRO	54	h	0.1000	173
HD2	54.456559589	33.804157775	71.264945954	PRO	54	h	0.1000	174
C	50.163700104	34.973747253	71.631501244	PRO	54	c'	0.3800	175
O	50.381851136	36.156935425	71.709472650	PRO	54	o'	−0.3800	176
CB	51.868888855	33.209735870	72.497510364	PRO	54	c2	−0.2000	177
HB1	51.140216527	33.071922302	73.319641113	PRO	54	h	0.1000	178
HB2	52.201725006	32.193626404	72.207275391	PRO	43	h	0.1000	179
CG	53.074722290	34.047100067	72.925216675	PRO	54	c2	−0.2000	180
HG1	52.742275235	34.920612335	73.520271301	PRO	54	h	0.1000	181
HG2	53.794158936	33.482940674	73.547462463	PRO	54	h	0.1000	182
N	48.950717926	34.451953345	71.882225037	GLY	55	h	−0.5000	183
CA	47.799011230	35.264900208	72.354157012	GLY	55	cg	0.2000	184
HN	48.913242340	33.427757262	71.533122253	GLY	55	hn	0.2800	185
HA1	47.529734802	36.291049957	71.943451445	GLY	55	h	0.1000	186
HA2	46.873668621	34.838525226	71.925056975	GLY	55	h	0.1000	187
C	47.624210355	35.262092590	73.898422241	GLY	55	c'	0.3800	188
O	47.184692353	34.228916165	74.411125183	GLY	55	o'	−0.3800	189
N	47.910079956	36.345748901	74.679351507	PRO	56	n	−0.4200	190
CA	47.789894104	36.319248199	76.162750244	PRO	56	ca	0.0600	191
HA	48.177116394	35.361667633	76.567420959	PRO	56	h	0.1000	192
CD	48.602729797	37.547939301	74.184952300	PRO	56	c2	0.0600	193
HD1	48.046642303	35.065422058	73.380996704	PRO	56	h	0.1000	194
HD2	49.595470428	37.261715750	73.794556152	PRO	56	h	0.1000	195
C	46.326736450	36.529109955	76.663719177	PRO	56	c'	0.3800	196
O	45.857757568	37.657508850	76.842526843	PRO	56	o'	4.2800	197
CB	48.777050018	37.430667577	76.578651428	PRO	56	c2	4.2000	198
HB1	48.524734497	37.895471832	77.549573352	PRO	56	h	0.1000	199
HB2	49.795513153	37.009433746	76.691307068	PRO	56	h	0.1000	200
CG	48.752750397	35.431644440	75.422836304	PRO	56	c2	−0.2000	201
HG1	47.879917145	39.105595450	75.522354126	PRO	56	h	0.1000	202
HG2	49.655212402	39.069591522	75.391311646	PRO	56	h	0.1000	203
N	45.616943359	35.415058136	76.897827148	HIS	57	n	−0.5000	204
HN	46.014194489	34.579135895	76.450401306	HIS	57	hn	0.2500	205
CA	44.212440491	35.434158325	77.393567493	HIS	57	ca	0.1100	206
HA	43.635601044	36.135776520	76.762800753	HIS	57	h	0.1000	207
C	44.146274567	35.852919312	75.904235540	HIS	57	c'	0.3800	208
O	44.715753815	35.174930573	79.742973325	HIS	57	o'	−0.3800	209
CB	43.577262578	34.025093079	77.198188782	HIS	57	c2	−0.2000	210
HB1	44.242053986	33.250709534	77.626457732	HIS	57	h	0.1000	211
HB2	42.665222165	33.964206696	77.822631536	HIS	57	h	0.1000	212
CG	43.190551758	33.606594056	75.770996094	HIS	57	c5	0.1000	213
HD1	44.099815216	33.724720001	74.654500415	HIS	57	np	−0.4200	214
CE1	43.220783234	33.103317261	73.724327087	HIS	57	c5	0.2700	215
HE1	42.000507355	32.603566577	74.087806702	HIS	57	np	0.5000	216
CD2	42.008693695	32.921348572	75.433784485	HIS	57	c5	0.0100	217
HE1	43.579235077	32.995296475	72.708923340	HIS	57	h	0.1300	218
HE2	41.324546514	32.061363220	73.538429260	HIS	57	hn	0.2500	219
HD2	41.238662720	32.635732910	76.138023376	HIS	57	h	0.1300	220
N	43.493415833	37.014365150	79.314620972	PRO	58	n	−0.4200	221
CA	43.576023102	37.521991730	80.713310242	PRO	58	ca	0.0600	222
HA	44.640773772	37.575522258	82.019523621	PRO	58	h	0.1000	233
CD	42.828823090	37.960189519	75.395561215	PRO	58	c2	0.0600	224
HD1	42.088195801	37.471439362	77.735352080	PRO	58	h	0.1000	225
HD2	43.573791504	35.467678070	77.749885559	PRO	58	h	0.1000	226
C	42.782455444	36.660888672	81.747703552	PRO	55	c'	0.2500	227
O	41.546211243	36.662254851	81.766304016	PRO	58	o'	−0.3800	228
CB	43.067096710	38.972381592	80.563407898	PRO	58	c2	−0.2000	229
HB1	42.553604126	39.355644226	81.465919495	PRO	58	h	0.1000	230
HB2	43.922630310	39.652961731	80.282720947	PRO	58	h	0.1000	231
CG	42.156223297	35.958541870	79.333923340	PRO	58	c2	−0.0200	232
HG1	41.146640778	38.599040985	79.611282349	PRO	58	h	0.1000	233
HG2	42.036239624	39.956802365	75.571780396	PRO	58	h	0.1010	234
N	43.511478424	35.934525351	82.617271423	ALA	59	n	−0.5000	235
HN	44.509765625	35.905010233	82.256589050	ALA	59	hn	0.2800	236
CA	42.913761139	34.928112030	83.544029236	ALA	59	ca	0.1200	237
HA	42.154701233	34.341350555	82.987930298	ALA	59	h	0.1000	238
C	42.151308746	35.481468201	84.521502656	ALA	59	c'	0.2800	239

TABLE NO. 2-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
O	42.459964752	35.112228394	85.965652466	ALA	59	o'	−0.3800	240
CB	44.063701630	33.948829651	83.858291626	ALA	59	c3	−0.3000	241
HB1	43.708641052	33.104522705	84.478195190	ALA	59	h	0.1000	242
HB2	44.502014160	33.503505707	82.944122314	ALA	59	h	0.1000	243
HB3	44.882900238	34.433902740	84.422813416	ALA	59	h	0.1000	244
N	41.178901672	36.333106995	84.577079773	ALA	60	n	−0.5000	245
HN	41.112052917	36.562049866	83.573013306	ALA	60	hn	0.2800	246
CA	40.189502716	36.803413391	85.578857422	ALA	60	ca	0.1200	247
HA	40.008514404	35.981742559	86.302818298	ALA	60	h	0.1000	248
C	38.811019597	37.037807465	84.860046387	ALA	60	c'	0.3800	249
O	37.881835938	36.301105499	85.205276489	ALA	60	o'	−0.3800	250
CB	40.746566772	37.985549927	86.401313782	ALA	60	c3	−0.3000	251
HB1	39.997776031	38.372333527	87.116531372	ALA	60	h	0.1000	252
HB2	41.624504089	37.670467377	86.996170044	ALA	60	h	0.1000	253
HB3	41.079444585	38.830841064	85.774902344	ALA	60	h	0.1000	254
N	38.609931946	37.922218323	83.826889035	PRO	61	n	−0.4200	255
CA	37.398490906	37.873073578	82.950836182	PRO	61	ca	0.0600	256
HA	36.485267638	37.839759827	83.576164246	PRO	61	h	0.1000	257
CD	39.352070615	39.001556396	83.460945129	PRO	61	c2	0.0600	258
HD1	40.590957642	38.653537750	83.311111430	PRO	61	h	0.1000	259
HD2	39.564567512	39.780746460	84.247795105	PRO	61	h	0.1000	260
C	37.256752532	36.700027466	81.909835515	PRO	61	c'	0.3800	261
O	36.243316650	36.657413483	81.209106445	PRO	61	o'	−0.3800	262
CB	37.477272034	39.256717652	82.271400452	PRO	61	c2	−0.2000	263
HB1	36.964195251	39.298637390	81.290786743	PRO	61	h	0.1000	264
HB2	36.951357574	40.016944885	82.906776425	PRO	61	h	0.1000	265
CG	38.972518921	39.562160492	82.163795471	PRO	61	c2	−0.2000	266
HG1	39.409160614	39.034259796	81.293624578	PRO	61	h	0.1000	267
HG2	39.183399200	40.640045166	82.032295227	PRO	61	h	0.1000	268
N	38.213741302	35.753643036	81.804443359	SER	62	n	−0.5000	269
CA	38.144962311	34.600208282	80.863403320	SER	62	ca	0.1200	270
HN	39.020967804	35.895751953	82.434890747	SER	62	hn	0.2800	271
HA	37.892318726	34.983673096	79.856529236	SER	62	h	0.1000	272
C	37.055021973	31.324326324	81.273231506	SER	62	c'	0.3800	273
O	37.352454436	32.625560760	82.070343018	SER	62	o'	0.3800	274
CB	39.569152532	33.994293213	80.760513306	SER	62	c2	−0.1700	275
HB1	39.601100922	33.242229462	79.949050903	SER	62	h	0.1000	276
HB2	40.217050934	34.759819031	80.475799561	SER	62	h	0.1000	277
CG	39.955038330	33.367904663	81.986091614	SER	62	oh	0.1000	278
HG	39.157264709	32.925077698	82.316406250	SER	62	ho	0.3500	279
N	35.853912254	33.643447876	80.748901367	SER	63	n	−0.5000	280
CA	34.681579590	32.893772125	81.280097961	SER	63	ca	0.1200	251
HN	35.734226227	34.507610321	80.200576782	SER	63	hn	0.2800	282
HA	34.978878021	32.281963345	82.158424377	SER	63	h	0.1000	283
C	34.028987885	31.963005066	80.214455165	SER	63	c'	0.3800	254
O	33.624385534	32.404701233	79.134857178	SER	63	o'	−0.3800	285
CB	33.674541473	33.937455035	81.815757751	SER	63	c2	−0.1700	286
HB1	34.172107697	34.614356995	82.538948059	SER	63	h	0.1000	287
HB2	33.303077695	34.594402313	81.003784180	SER	63	h	0.1000	238
CG	31.576236725	33.301517487	82.471984863	SER	63	oh	−0.3800	289
HG	32.084590912	32.806625366	81.807708740	SER	63	ho	0.3500	290
N	33.852622986	30.677625656	80.556045532	TRP	64	n	−0.5000	291
CA	33.070865631	29.709415436	79.732810974	TRP	64	ca	0.1200	292
HN	34.153343201	30.435497254	81.506057739	TRP	64	hn	0.2800	293
HA	33.375720978	29.840501785	78.676765442	TRP	64	h	0.1000	294
C	31.526346207	29.958730698	79.816452026	TRP	64	c'	0.3800	295
O	30.936735153	29.911306351	80.900970459	TRP	64	o'	−0.3800	296
CB	33.498536517	25.253648758	80.086425751	TRP	64	c2	−0.2000	297
HB1	32.936897276	27.550512314	79.442245483	TRP	64	h	0.1000	298
HB2	34.548812566	28.110004426	79.767990112	TRP	64	h	0.1000	299
CG	33.372635702	27.788063049	81.551361084	TRP	64	c5	0.0000	300
CD1	32.236022969	27.198472977	82.145393372	TRP	64	c5	0.0100	301
HE1	32.442039490	26.926628113	83.513259888	TRP	64	np	−0.5000	302
CE2	33.734771729	27.365074158	83.750877380	TRP	64	c5	0.1100	303
CD2	34.313194275	27.855219574	82.565856934	TRP	64	c5	0.0000	304
HD1	31.305589935	27.011001587	81.622375485	TRP	64	h	0.1000	305
HE1	31.781488419	26.532098770	84.192108154	TRP	64	hn	0.2800	306
CE3	35.633460999	28.410655975	85.581642151	TRP	64	cp	−0.1000	307
HE3	36.086208344	25.812973022	81.656943054	TRP	64	h	0.1000	308
CZ3	36.338203430	28.401332855	83.786201477	TRP	64	cp	−0.1000	309
HZ3	37.342418671	28.800062180	83.816750090	TRP	64	h	0.1000	310
CH2	35.766292572	27.887487411	84.956939697	TRP	64	cp	−0.1000	311
HH2	36.336753845	27.895225525	85.875114441	TRP	64	h	0.1000	312
CZ2	34.469055176	27.367534637	84.959693909	TRP	64	cp	−0.1000	313
HZ2	34.033626556	26.978825430	85.868453979	TRP	64	h	0.1000	314

TABLE NO. 2-continued

Atom name	x	y	z	residue name and no.		atom type, charge and no.		
N	30.884126663	30.253189087	78.672058105	GLY	65	n	−0.5000	315
CA	29.433704376	30.522933426	78.625785828	GLY	54	cg	0.0200	316
HN	31.490442276	30.324731827	77.837860107	GLY	65	hn	0.2800	317
HA1	29.049486160	30.927183151	79.604759216	GLY	65	h	0.1000	318
HA2	29.301883698	31.462034225	77.967575073	GLY	65	h	0.1000	319
C	28.566276550	29.436250687	78.049354553	GLY	65	c'	0.3800	320
O	28.476503372	29.383417130	76.820671082	GLY	65	o'	−0.3800	321
N	27.919076920	28.520057678	78.830680847	PRO	66	n	−0.4200	322
CA	27.254582451	27.307062149	78.266265869	PRO	66	ca	0.0600	323
HA	28.007204056	26.749544144	77.674018860	PRO	66	h	0.1000	324
CD	27.931732178	28.547025681	80.307373047	PRO	66	c2	0.0600	325
MD1	27.674114227	29.536535263	80.727989197	PRO	66	h	0.1000	326
MD2	28.930458069	28.264209747	80.693565369	PRO	66	h	0.1000	327
C	25.991449356	27.540506363	77.367637634	PRO	66	c'	0.3800	328
O	25.234470367	28.498056412	77.550445557	PRO	66	o'	−0.3800	329
CB	26.947065353	26.487516403	79.540168762	PRO	66	c2	−0.2000	330
HB1	26.021696091	25.883453369	79.667781067	PRO	66	h	0.1000	331
HB2	27.765922546	25.768106561	79.730659485	PRO	66	h	0.1000	332
CG	26.879997253	27.505201340	80.680580139	PRO	66	c2	−0.2000	333
HG1	25.878761292	27.974497401	80.712356567	PRO	66	h	0.1000	334
HG2	27.057765961	27.053478241	81.674545288	PRO	66	h	0.1000	335
N	25.764133453	26.624628067	76.406608582	CYS	67	n	−0.5000	336
CA	24.578670502	26.665664673	75.512832642	CYS	67	ca	0.1200	337
HN	26.474828720	25.893449783	76.320098877	CYS	67	hn	0.2800	338
HA	24.437746048	27.701400757	75.152099609	CYS	67	h	0.1000	339
C	23.256376266	26.130277634	76.174392700	CYS	67	c'	0.3800	340
O	23.219629288	24.940492630	76.516906738	CYS	67	o'	−0.3800	341
CB	24.900175095	25.819908432	74.260444641	CYS	67	c2	−0.3000	342
HB1	25.807971954	26.178848267	73.749794106	CYS	67	h	0.1000	343
HB2	25.105169296	24.761932373	74.532623291	CYS	67	h	0.1000	344
CG	23.472158432	25.844451904	73.133270264	CYS	67	s1	0.1000	345
N	22.124137878	26.895683289	76.264381409	PRO	68	n	−0.4200	346
CA	20.786550522	26.297697067	76.829830933	PRO	69	ca	0.0600	347
HA	20.877141953	25.506265650	77.300582886	PRO	68	h	0.1000	348
CD	22.161409378	28.364057541	76.432929993	PRO	68	c2	0.0600	349
HB1	22.628620148	28.901371002	75.585960388	PRO	68	h	0.1000	350
HB2	22.732339859	28.631174088	77.345329285	PRO	68	h	0.1000	351
C	20.190311432	25.593938828	75.255737305	PRO	68	c'	0.3800	352
O	20.566764832	24.451507568	74.984413147	PRO	68	o'	−0.3800	353
CB	20.033475876	27.483673096	77.173645020	PRO	68	c2	−0.2000	354
HB1	18.940057755	27.449979782	77.017181396	PRO	68	h	0.1000	355
HB2	20.183664322	27.458950043	78.271354675	PRO	68	h	0.1000	356
CG	20.687761307	28.746078491	76.604209900	PRO	68	c2	−0.2000	357
HG1	20.234010696	29.017192841	75.632743835	PRO	68	h	0.1000	358
HG2	20.559530258	29.623554230	77.265640259	PRO	68	h	0.1000	359
N	19.297069550	26.226366043	74.460205078	ARG+	69	n	−0.5000	360
CA	18.727945328	25.594141006	73.229873657	ARG+	69	ca	0.1200	361
HN	18.899488449	27.074655533	74.874603271	ARG+	69	hn	0.2800	362
HA	19.468439102	24.889890671	72.798027039	ARG+	69	h	0.1000	363
C	18.426181793	26.666866302	72.127845764	ARG+	69	c'	0.3800	364
O	17.302417755	27.170328140	72.057907104	ARG+	69	o'	−0.3800	365
CB	17.487716675	24.741283417	73.645401001	ARG+	69	c2	−0.2000	366
HB1	17.790594101	24.038330078	74.447227478	ARG+	69	h	0.1100	367
HB2	16.742151260	25.409061432	74.119949341	ARG+	69	h	0.1100	368
CG	16.806570053	23.930654526	72.510940552	ARG+	69	c2	−0.2000	369
HG1	16.500089645	24.624885559	71.702163696	ARG+	69	h	0.1300	370
HG2	17.539186478	23.235509872	72.053100586	ARG+	69	h	0.1300	371
CD	15.574314117	23.148860931	73.007453918	ARG+	69	c2	−0.0900	372
HD1	15.890284538	27.374292374	73.738624573	ARG+	69	h	0.1300	373
HD2	14.902976036	23.843069077	73.554016113	ARG+	69	h	0.1300	374
HZ	14.865127563	22.521680832	71.855873108	ARG+	69	n1	−0.5000	375
HE	15.293711662	22.507183075	70.926025391	ARG+	69	hn	0.3600	376
CZ	13.645489693	21.980854034	71.902374268	ARG+	69	cr	0.4500	377
NH1	13.127552986	21.522832870	70.798370361	ARG+	69	n2	−0.5000	378
NH11	13.689088821	21.608518620	69.948852539	ARG+	69	hn	0.3600	379
NH12	12.188611031	21.122539520	70.853851318	ARG+	69	hn	0.3600	380
HN2	12.936479568	21.886768341	73.000465393	ARG+	69	n2	−0.500	381
NH21	12.008401871	21.462900162	72.952354431	ARG+	69	hn	0.3600	382
NH22	13.405644417	22.251142502	73.831558228	ARG+	69	hn	0.3600	383
N	19.430337904	26.966600418	71.273384094	ARG+	70	n	−0.5000	384
CA	19.316965103	27.784936905	70.016807556	ARG+	70	ca	0.1200	385
HW	20.317523956	26.522159576	71.527793884	ARG+	70	hn	0.2800	386
HA	19.139877319	27.013025284	69.241798401	ARG+	70	h	0.1000	387
C	20.690151215	28.397680283	69.573341370	ARG+	70	c'	0.3800	385
O	21.267679214	27.963806152	68.579154968	ARG+	70	o'	−0.3800	389

TABLE NO. 2-continued

Atom name	x	y	z	residue name and no.	atom type, charge and no.
CB	18.103752136	28.753581454	69.796676636	AR0+ 70	c2 −0.2000 390
HB1	18.134477615	29.133897751	68.754875153	ARG+ 70	h 0.1100 391
HB2	17.153977127	25.135446545	69.816154480	ARG+ 70	h 0.1100 392
CG	17.944366455	29.552959061	70.767364502	ARG+ 70	c2 −0.2000 393
HG1	18.201717377	29.630409241	71.795295715	ARG+ 70	h 0.1300 394
HG2	18.686578751	30.737569809	70.523498535	ARG+ 70	h 0.1300 395
CD	16.516407013	30.528032303	70.757499695	ARG+ 70	c2 −0.0900 396
HD1	16.205293655	30.812314987	69.732495169	ARG+ 70	h 0.1300 397
HD2	15.804359436	29.724859238	71.041206360	ARG+ 70	h 0.1300 398
HE	16.396289825	31.632033883	71.751823425	ARG+ 70	n1 −0.5000 399
NE	16.351929398	31.418577194	72.754444790	ARG+ 70	hn 0.3600 400
CE	16.270032883	32.931114197	71.475357056	ARG+ 70	cr 0.4500 401
HN1	16.119342804	33.758121490	72.470024109	ARG+ 70	n2 −0.5000 402
HN11	16.097789764	33.352241516	73.407394409	ARG+ 70	hn 0.3600 403
HN12	16.020824432	34.751869202	72.242614746	ARG+ 70	hn 0.3600 404
NH2	16.295974731	33.427280426	70.262794495	ARG+ 70	n2 −0.5000 405
HN21	16.192591263	34.436058562	70.142570496	ARG+ 70	hn 0.3600 406
HN22	16.439620972	32.732715607	69.527351379	ARG+ 70	hn 0.3600 407
N	21.215826035	29.506198883	70.104598999	TYRC 71	n −0.5000 408
HN	21.692993164	29.961484909	69.319816589	TYRC 71	hn 0.2800 409
CA	21.037544250	29.469150543	71.348726365	TYRC 71	ca 0.1300 410
HA	22.727062225	28.601654053	71.296867371	TYRC 71	h 0.1000 411
C	21.230804443	29.295030594	72.663772583	TYRC 71	c' 0.4100 412
CKT	20.420524597	30.105148315	73.113685608	TYRC 71	o' −0.3800 413
C	21.522385052	28.107995987	73.273979187	TYRC 71	ch −0.3800 414
HO	20.994127274	28.000011444	74.066841125	TYRC 71	ho 0.3500 415
CE	22.938613892	30.740638733	71.402084351	TYRC 71	c2 0.2000 416
HB1	22.321226120	31.652799606	71.283157349	TYRC 71	h 0.1000 417
HB2	23.363500595	30.553305817	72.420455933	TYRC 71	h 0.1000 418
CG	24.110603333	30.760416031	70.402580261	TYRC 71	cp 0.000 419
CD1	23.933057785	31.274461746	69.111869512	TYRC 71	cp −0.1000 420
HD1	22.977622986	31.679259164	68.509974670	TYRC 71	h 0.1000 421
CE1	24.985538483	31.264329910	68.201301575	TYRC 71	cp −0.1000 422
HE1	24.833002090	31.650396347	67.203536987	TYRC 71	h 0.1000 423
CZ	26.227394104	30.757091522	68.577156584	TYRC 71	cp 0.0300 424
OH	27.265548160	30.762763977	67.686424255	TYRC 71	oh −0.3800 425
HN	29.966634750	31.154350798	66.863937378	TYRC 71	ho 0.3500 426
CE2	26.415199280	30.251981735	69.859985352	TYRC 71	cp −0.1000 427
HE2	27.377700806	29.852491379	70.147377014	TYRC 71	h 0.1000 428
CD2	25.360828400	30.253871918	70.770927429	TYRC 71	cp −0.1000 429
HD2	25.521846771	29.846044540	71.760574341	TYRC 71	h 0.1000 430

*The numbering of the amino acids is shifted by minus 6 relative to the sequence SEQ ID No. 1

SEQUENCE LISTING

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(1) GENERAL INFORMATION:

   (iii) NUMBER OF SEQUENCES: 8


(2) INFORMATION FOR SEQ ID NO: 1:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 476 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

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

Leu Gln Pro Gly Ala Glu Val Pro Val Val Trp Ala Gln Glu Gly Ala
1               5                   10                  15

Pro Ala Gln Leu Pro Cys Ser Pro Thr Ile Pro Leu Gln Asp Leu Ser
            20                  25                  30
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Leu Leu Arg Arg Ala Gly Val Thr Trp Gln His Gln Pro Asp Ser Gly
        35                  40                  45

Pro Pro Ala Ala Ala Pro Gly His Pro Leu Ala Pro Gly Pro His Pro
    50                  55                  60

Ala Ala Pro Ser Ser Trp Gly Pro Arg Pro Arg Arg Tyr Thr Val Leu
65                  70                  75                  80

Ser Val Gly Pro Gly Gly Leu Arg Ser Gly Arg Leu Pro Leu Gln Pro
                85                  90                  95

Arg Val Gln Leu Asp Glu Arg Gly Arg Gln Arg Gly Asp Phe Ser Leu
            100                 105                 110

Trp Leu Arg Pro Ala Arg Arg Ala Asp Ala Gly Glu Tyr Arg Ala Ala
        115                 120                 125

Val His Leu Arg Asp Arg Ala Leu Ser Cys Arg Leu Arg Leu Arg Leu
    130                 135                 140

Gly Gln Ala Ser Met Thr Ala Ser Pro Pro Gly Ser Leu Arg Ala Ser
145                 150                 155                 160

Asp Trp Val Ile Leu Asn Cys Ser Phe Ser Arg Pro Asp Arg Pro Ala
                165                 170                 175

Ser Val His Trp Phe Arg Asn Arg Gly Gln Gly Arg Val Pro Val Arg
            180                 185                 190

Glu Ser Pro His His His Leu Ala Glu Ser Phe Leu Phe Leu Pro Gln
        195                 200                 205

Val Ser Pro Met Asp Ser Gly Pro Trp Gly Cys Ile Leu Thr Tyr Arg
    210                 215                 220

Asp Gly Phe Asn Val Ser Ile Met Tyr Asn Leu Thr Val Leu Gly Leu
225                 230                 235                 240

Glu Pro Pro Thr Pro Leu Thr Val Tyr Ala Gly Ala Gly Ser Arg Val
                245                 250                 255

Gly Leu Pro Cys Arg Leu Pro Ala Gly Val Gly Thr Arg Ser Phe Leu
            260                 265                 270

Thr Ala Lys Trp Thr Pro Pro Gly Gly Gly Pro Asp Leu Leu Val Thr
        275                 280                 285

Gly Asp Asn Gly Asp Phe Thr Leu Arg Leu Glu Asp Val Ser Gln Ala
    290                 295                 300

Gln Ala Gly Thr Tyr Thr Cys His Ile His Leu Gln Glu Gln Gln Leu
305                 310                 315                 320

Asn Ala Thr Val Thr Leu Ala Ile Ile Thr Val Thr Pro Lys Ser Phe
                325                 330                 335

Gly Ser Pro Gly Ser Leu Gly Lys Leu Leu Cys Glu Val Thr Pro Val
            340                 345                 350

Ser Gly Gln Glu Arg Phe Val Trp Ser Ser Leu Asp Thr Pro Ser Gln
        355                 360                 365

Arg Ser Phe Ser Gly Pro Trp Leu Glu Ala Gln Glu Ala Gln Leu Leu
    370                 375                 380

Ser Gln Pro Trp Gln Cys Gln Leu Tyr Gln Gly Glu Arg Leu Leu Gly
385                 390                 395                 400

Ala Ala Val Tyr Phe Thr Glu Leu Ser Ser Pro Gly Ala Gln Arg Ser
                405                 410                 415

Gly Arg Ala Pro Gly Ala Leu Pro Ala Gly His Leu Leu Leu Phe Leu
            420                 425                 430

Thr Leu Gly Val Leu Ser Leu Leu Leu Leu Val Thr Gly Ala Phe Gly
        435                 440                 445

Phe His Leu Trp Arg Arg Gln Trp Arg Pro Arg Arg Phe Ser Ala Leu
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    450                 455                 460

Glu Gln Gly Ile His Pro Arg Arg Leu Arg Ala Arg
465                 470                 475


(2) INFORMATION FOR SEQ ID NO: 2:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 32 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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

GCGCCTCGAG GCCCAGACCA TAGGAGAGAT GT                                    32


(2) INFORMATION FOR SEQ ID NO: 3:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 40 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

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

GCGCAGATCT CTCCAGACCC AGAACAGTGA GGTTATACAT                            40


(2) INFORMATION FOR SEQ ID NO: 4:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 34 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GCGCAGATCT ACCTGGGCTA GACAGCTCTG TGAA                                  34


(2) INFORMATION FOR SEQ ID NO: 5:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 37 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGCCGTCGAC CGCTGCCCAG ACCATAGGAG AGATGTG                               37


(2) INFORMATION FOR SEQ ID NO: 6:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 37 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCGCGTCGAC TTACATCGAG GCCTGGCCCA GGCGCAG                               37
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(2) INFORMATION FOR SEQ ID NO: 7:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 35 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCGCGTCGAC TTAACCCAGA ACAGTGAGGT TATAC                              35


(2) INFORMATION FOR SEQ ID NO: 8:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 35 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GCGCGTCGAC TTAACCTGGG CTAGACAGCT CTGTG                              35
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We claim:

[**1**. A soluble polypeptide fraction consisting of at least one of the 4 immunoglobulin type extra-cellular domains of the LAG-3 protein (amino acids 1 to 4, 5 to 239, 240 to 330 and 331 to 412 of sequence SEQ ID NO:1), wherein one or more arginine (Arg) residues at positions 73, 75 and 76 of SEQ ID NO:1 are substituted with glutamic acid (Glu) and said at least one extra-cellular domain of LAG-3 protein is optionally fused to a supplementary peptide sequence as a fusion protein.]

2. A soluble polypeptide [fraction] according to claim [**1**] *11*, further bound to a toxin or a radioisotope.

[**3**. A soluble polypeptide fraction according to claim **1**, further bound to a toxin or a radioisotope wherein said supplementary peptide sequence is present and comprises a portion of an immunoglobulin.]

4. A soluble polypeptide [fraction] according to claim [**3**] *13*, wherein said supplementary peptide sequence comprises a portion of an immunoglobulin of IgG4 isotype.

5. A method for producing the soluble polypeptide [fraction] of claim [**1**] *11*, which soluble polypeptide [fraction] further comprises a portion of an immunoglobulin, comprising [the steps of]:

- inserting a DNA molecule comprising a fusion of fragments of cDNA coding for the polypeptide regions corresponding to LAG-3 or derived from LAG3 with cDNA coding for the portion of the immunoglobulin;
- transfecting the DNA molecule into a host expression system; and
- producing the soluble polypeptide [fraction] by expression in the host.

[**6**. A soluble polypeptide fraction consisting of at least one of four immunoglobulin-type extracellular domains of LAG-3 protein corresponding to amino acid residues 1–4, 5–239, 240–330, and 331–412 of SEQ ID NO:1, fused to a supplementary peptide sequence as a fusion protein.]

[**7**. A soluble polypeptide fraction according to claim **6**, further bound to a toxin or a radioisotope.]

[**8**. A soluble polypeptide fraction according to claim **6**, further bound to a toxin or a radioisotope wherein said supplementary peptide sequence comprises a portion of an immunoglobulin.]

[**9**. A soluble polypeptide fraction according to claim **8**, wherein said supplementary peptide sequence comprises a portion of an immunoglobulin of IgG4 isotype.]

[**10**. A method for producing the soluble polypeptide fraction of claim **6**, which soluble polypeptide fraction further comprises a portion of an immunoglobulin, comprising the steps of:

- inserting a DNA molecule comprising a fusion of fragments of cDNA coding for the polypeptide regions corresponding to LAG-3 with cDNA coding for the portion of the immunoglobulin;
- transfecting the DNA molecule into a host expression system; and
- producing the soluble polypeptide fraction by expression in the host.]

11. A soluble polypeptide comprising a first immunoglobulin type extracellular domain of LAG-3 protein (*amino acid residues 1–159 of SEQ ID NO:1*), *wherein one or more arginine* (*Arg*) *residues at positions 73, 75 and 76 of SEQ ID NO:1 are substituted with glutamic acid* (*Glu*).

12. A soluble polypeptide according to claim 11, further comprising a supplementary peptide sequence fused to said first extracellular domain of LAG-3 protein as a fusion protein.

13. A soluble polypeptide according to claim 12, wherein said supplementary peptide sequence comprises a portion of an immunoglobulin.

14. A soluble polypeptide according to claim 11, wherein said first extracellular domain of LAG-3 protein is fused to one or more additional immunoglobulin type extracellular domains of LAG-3 protein selected from the group consisting of amino acid residues 160–239, 240–330, and 331–412 of SEQ ID NO:1.

15. A soluble polypeptide according to claim 14, further comprising a supplementary peptide sequence fused to either said first extracellular domain of LAG-3 protein or one of said one or more additional extracellular domains of LAG-3 protein.

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