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(54) Title: BLOCKING INTERCELLULAR INTERACTIONS WITH CD43 CHIMERIC MOLECULES (57) Abstract Disclosed is a method of inhibiting an interaction between a first and a second cell in a mammal. The method involves providing a soluble chimeric molecule which includes: a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of the first cell covalently bonded to b) a CD43 extracellular domain bearing a net negative charge at physiological pH; and administering the chimeric molecule to the mammal to inhibit the intercellular interaction. Also disclosed are useful CD43-containing chimeric molecules.		

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BLOCKING INTERCELLULAR INTERACTIONSWITH CD43 CHIMERIC MOLECULESBackground of the Invention

5 This invention relates to methods of inhibiting cellular interactions, particularly interactions leading to inflammation or pathogenic infection.

 One important step in the generation of cellular immune responses is the adherence of lymphocytes to each
10 other or to other cells of the immune system. Several molecular pathways for these cell-cell adhesive interactions have been defined (Springer, *Nature* 246:425-434, 1990), and among lymphocytes, the binding of lymphocyte function-associated antigen 1 (LFA-1) to its
15 counterreceptor, intercellular adhesion molecule 1 (ICAM-1) (Rothlein, R. et al., *J. Immunol.*, 137:1270-1274, 1986; Marlin, S.D. et al., *Cell*, 51:813-819, 1987) constitutes a dominant adhesion pathway. Antibodies that prevent interaction of these counterreceptors can block
20 homotypic adhesion (Mentzer, S.J. et al. *J. Immunol.*, 135:9-11, 1985; Rothlein, R. et al., *J. Exp. Med.*, 163:1132-1149, 1986) and T-lymphocyte-mediated killing (Davignon, D. et al., *J. Immunol.*, 127:590-595, 1981; Sanchez-Madrid, F. et al., *J. Exp. Med.*, 158:1785-1803,
25 1983), suggesting the importance of this adhesion pathway for normal immunity. Moreover, genetic deficiency of the β subunit of LFA-1 results in an immunodeficiency characterized by recurrent life-threatening bacterial infections and severe defects in adhesion-dependent
30 leukocyte functions (Anderson, D.C. et al., *J. Infect. Dis.*, 152:668-689, 1985; Arnaout, M. A. et al., *J. Clin. Invest.*, 74:1291-1300, 1984; Ross, G.D. et al., *Blood*, 66:882-890, 1985).

 Regulatory mechanisms that enhance LFA-1 binding
35 to ICAM-1 recently have been described (Dustin, M.L. et

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al., *J. Cell Biol.*, 107:321-331, 1988; Dustin, M.L. et al., *Nature (London)*, 341:619-624, 1989) and likely function to promote purposeful interactions between different types of lymphocytes as well as between
5 lymphocytes and nonimmune cells. For example, lymphocyte binding to endothelium is augmented by preexposure of the endothelial cells to inflammatory cytokines, and the enhanced adhesion is due, in part, to a quantitative increase in ICAM-1 expression by the endothelial cells
10 (Dustin, M.L. et al., *J. Cell Biol.*, 107:321-331, 1988). Alternatively, activation of T lymphocytes by T-cell receptor crosslinking results in a marked but transient enhancement of lymphocyte LFA-1 avidity for ICAM-1, without increasing levels of LFA-1 expression (Dustin,
15 M.L. et al., *Nature (London)*, 341:619-624, 1989).

Regulation of lymphocyte adhesion can also be viewed as involving inhibitory elements that limit inconsequential cell-cell interactions. In this context, the extensively sialylated (and thus highly negatively
20 charged) lymphocyte membrane glycocalyx likely plays a role (Bell, G.I. et al., *Biophys. J.*, 45:1051-1064, 1984). It has been proposed that the negatively charged glycocalyx repulses cells bearing like charge and, thus, functions to limit cell-cell adhesive interactions
25 (Brown, W.R.A. et al., *Nature (London)*, 289:456-460, 1981). CD43 (sialophorin or leukosialin) is a candidate molecule to regulate adhesion between lymphocytes because it is a major integral membrane component of the lymphocyte glycocalyx (Brown, W.R.A. et al., *Nature*
30 *(London)*, 289:456-460, 1981; Remold-O'Donnell et al., *J. Biol. Chem.*, 261:7526-7530, 1986; Carlsson, S.R. et al., *J. Biol. Chem.*, 261:12779-12786, 1986).

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Summary of the Invention

Applicant has discovered that the CD43 protein, when presented on a cell surface, blocks interaction of that CD43-bearing cell with other cells which present
5 either CD43 or very likely any other negatively-charged protein on their cell surfaces. Because the CD43 inhibitory effect appears to be relatively non-specific, any number of therapeutic CD43 chimeric molecules may be constructed and used to disrupt deleterious intercellular
10 interactions. Such chimeras include two functional domains: (a) a cell targeting domain which directs specific binding of the chimeric molecule to a molecule (e.g., a receptor, ligand, or counterreceptor) on the surface of one or both of the cells whose interaction is
15 to be disrupted and (b) a CD43 extracellular domain bearing a net negative charge at physiological pH which antagonizes the intercellular interaction.

In one particular example, CD43 chimeric molecules can be used to disrupt the intercellular interactions
20 leading to inflammation. For example, a negatively charged CD43 extracellular domain may be covalently bonded to the leukocyte cell surface molecules lacto-N-fucopentaose III (LNF III) or sialyl Lewis^x. These molecules bind specifically to the receptors P-selectin
25 (PADGEM, GMP140, CD62) and ELAM-1 (respectively), proteins which are expressed on the surface of endothelial cells which are activated during an inflammatory response (Larsen et al., *Cell* 63:467, 1990; Johnston et al., *Cell* 56:1033, 1989; Aruffo et al., *Cell*
30 67:35, 1991, and Walz et al., *Science* 250:1132, 1990). Upon administration of CD43:LNF III or CD43:sialyl Lewis^x, these chimeric proteins bind and "coat" the endothelial cell surface, blocking interaction between the endothelium and the immune cells -- both by blocking
35 access of the immune cell ligand to the endothelial cell

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receptor and also by non-specifically repelling the immune cell, probably as a result of CD43's high negative charge. By inhibiting the endothelial cell-immune cell adhesive interaction, the therapeutic proteins of the invention reduce or eliminate inflammation.

Alternatively, a negatively charged CD43 extracellular domain may be fused to the receptors VCAM-1 and ICAM-1 to produce chimeric proteins CD43:VCAM-1 and CD43:ICAM-1; such proteins bind and coat the surface of certain types of leukocytes, blocking interaction with their endothelial counterreceptors and therefore the intracellular interaction between the immune cells and the endothelium which leads to inflammation.

CD43 chimeras are also useful for inhibiting interactions necessary for pathogenic infection. For example, both rhinovirus and the malarial *Plasmodium* protozoans have been shown to bind ICAM-1 and thus a CD43:ICAM-1 chimeric molecule which blocks rhinovirus or *Plasmodium* binding may prove to be a useful therapeutic for treating or preventing the common cold or malaria. In another example, a CD43:CD4 chimera can be used to coat the exterior of the human immunodeficiency virus, thereby interfering with host cell infectivity. A similar approach using a negatively charged CD43 extracellular domain covalently linked to a toxin molecule (e.g., a *Staphylococcus* or *E. coli* toxin) can be used to inhibit toxin effects.

In another general approach, a negatively charged CD43 extracellular domain may be covalently linked to an antibody molecule which is specific for a particular cell surface molecule. In one example, the extracellular CD43 domain may be linked to a published anti-PADGEM antibody (see, e.g., Larsen et al., *Cell* 63:467, 1990) to produce a CD43 chimeric molecule which blocks binding of

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leukocytes to endothelium and thereby prevents inflammation (as described above).

Accordingly, in general, the invention features a method of inhibiting an interaction between a first and a
5 second cell in a mammal, involving providing a soluble chimeric molecule which includes (a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of the first cell covalently bonded to (b) a CD43 extracellular domain
10 bearing a net negative charge at physiological pH; and administering the chimeric molecule to the mammal to inhibit the intercellular interaction.

In preferred embodiments, the second cell bears a negatively-charged molecule (for example, CD43) on its
15 cell surface; the CD43 extracellular domain is glycosylated; the CD43 extracellular domain includes the N-terminal 180 amino acids of the CD43 protein sequence; the cell targeting molecule is a P-selectin-binding portion of an LNF III receptor protein; the cell
20 targeting molecule is an ELAM-1-binding portion of a sialyl Lewis^x receptor protein; the cell targeting molecule is an LFA-1-binding portion of an ICAM-1 receptor protein; the cell targeting molecule is a VLA-4-binding portion of a VCAM-1 receptor protein; the cell
25 targeting molecule is a human immunodeficiency virus-binding portion of CD4; the cell targeting molecule is a rhinovirus-binding portion of ICAM-1; the cell targeting molecule is a *Plasmodium*-binding portion of ICAM-1; and the first cell is an activated endothelial cell or an
30 infective pathogen (for example, rhinovirus, *Plasmodium falciparum*, *Plasmodium malariae*, or a human immunodeficiency virus).

In a second aspect, the invention features a chimeric molecule which includes (a) a cell targeting
35 molecule which is capable of specifically recognizing and

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binding to a molecule on the surface of a cell joined to
(b) a CD43 extracellular domain bearing a net negative
charge at physiological pH.

In preferred embodiments, the CD43 extracellular
5 domain is glycosylated; the CD43 extracellular domain
includes the N-terminal 180 amino acids of the CD43
protein sequence; the cell targeting molecule is a P-
selectin-binding portion of an LNF III receptor protein;
the cell targeting molecule is an ELAM-1-binding portion
10 of a sialyl Lewis^x receptor protein; the cell targeting
molecule is an LFA-1-binding portion of an ICAM-1
receptor protein; the cell targeting molecule is a VLA-4-
binding portion of a VCAM-1 receptor protein; the cell
targeting molecule is a human immunodeficiency virus-
15 binding portion of CD4; the cell targeting molecule is a
rhinovirus-binding portion of ICAM-1; the cell targeting
molecule is a Plasmodium-binding portion of ICAM-1; the
cell targeting molecule specifically recognizes and binds
to a molecule on the surface of an activated endothelial
20 cell; and the cell targeting molecule specifically
recognizes and binds to an infective pathogen.

In a third aspect, the invention features a method
of reducing inflammation in a mammal, which involves
providing a chimeric molecule which includes (a) a cell
25 targeting molecule which is capable of specifically
recognizing and binding to a molecule on the surface of
an activated endothelial cell joined to (b) a CD43
extracellular domain bearing a net negative charge at
physiological pH; and administering the chimeric molecule
30 to the mammal to inhibit intercellular interaction
between the activated endothelial cell and white blood
cells.

In preferred embodiments, the cell targeting
molecule is a P-selectin-binding portion of an LNF III
35 receptor protein; and the cell targeting molecule is an

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ELAM-1-binding portion of a sialyl Lewis^x receptor protein.

In a fourth aspect, the invention features a method of treating pathogen infection in a mammal, which involves providing a chimeric molecule which includes (a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of the pathogen joined to (b) a CD43 extracellular domain bearing a net negative charge at physiological pH; and administering the chimeric molecule to the mammal to inhibit intercellular interaction between the pathogen and the mammalian cell it normally infects.

By a "cell targeting molecule" is meant a molecule which binds with specific and high affinity to a binding partner present on the surface of the cell whose intercellular interaction one desires to inhibit. Such a cell targeting molecule includes, without limitation, any cell surface ligand, receptor, counterreceptor, cell adhesion molecule, enzyme, or substrate, or any antibody which is specific for a molecule on the surface of the target cell. The cell targeting molecule may be proteinaceous or non-proteinaceous (for example, a non-peptide cofactor or a carbohydrate recognized by a cell adhesion molecule). Similarly, the cell targeting molecule may bind to a proteinaceous or non-proteinaceous molecule on the target cell surface.

By "negatively charged" is meant that the cell surface molecule bears a negative charge at physiological pH.

By an "activated endothelial cell" is meant that the endothelial cell, in response to antigen stimulation, has initiated the cellular events (e.g., expression of cell surface proteins) characteristic of an inflammatory response.

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The CD43 chimeric molecules of the instant invention provide a number of therapeutic advantages. For example, the specificity with which the CD43 chimeric molecule interacts with its appropriate cell surface binding partner allows targeting of the therapeutic protein to the affected area (e.g., the inflamed endothelium); such targeted therapy allows highly effective inhibition of deleterious cell interactions with minimal side effects. In two particular examples, the CD43:LNF III and CD43:sialyl Lewis^x chimeric molecules bind receptors present in significant numbers on inflamed, but not healthy, vascular endothelium. Upon administration, CD43:LNF III and CD43:sialyl Lewis^x accumulate at the site of vascular damage, maximizing the potency of the inflammation treatment without affecting healthy vascular endothelium and importantly without generally immunosuppressing the patient.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

Detailed Description

Description of the Drawings

FIG. 1 is a series of graphs showing CD43 expression by HeLa cell transfectants and human T lymphocytes. Cells were stained either with control normal mouse sera (*Left*) or the anti-CD43 monoclonal antibody anti-Leu 22 (*Right*) as described herein. (a) CD43-negative HeLa cells. (b) CD43-positive HeLa cells. (c) PHA-activated human T lymphocytes. The level of CD43 expression by the phytohemagglutinin (PHA)-treated T lymphocytes is representative of the level of expression in peripheral blood T cells and all T-cell lines used in the experiments described herein.

FIG. 2 is a series of bar graphs showing the effect of CD43 on T-lymphocyte adhesion to HeLa cell

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transfectants. Carboxyfluorescein-labeled lymphocytes were allowed to adhere to HeLa cell transfectants in microtiter wells as described herein. Human lymphocytes tested were peripheral blood T lymphocytes (PBL-T); PHA-
5 activated T cells maintained in IL-2 (PHA-T); thymocytes (THY); a T-cell lymphoma line (SupT1); a human T-lymphotropic virus type 1-transformed T-cell line (C91/PL); and an IL-2-dependent T-cell clone (JL89). For this and all subsequent figures, each bar represents the
10 mean percentage of bound lymphocytes from three experiments $\pm$ SEM.

FIG. 3 is a series of bar graphs showing the effect of CD43 on LFA-1-mediated T-lymphocyte adhesion. Lymphocytes were preincubated with either normal mouse
15 sera, or monoclonal antibody ascites to LFA-1 (TS1/22) or to CD2 (TS2/18) (all at 1:400 dilution) for 30 min on ice and then allowed to adhere to HeLa cell transfectants for 30 min at room temperature. (A) Binding of the JL89 T-cell clone to the HeLa cell transfectants. (B) Binding
20 of PHA-T cells to the HeLa cell transfectants.

FIG. 4 is a series of bar graphs showing the effect of CD43 on adhesion of PMA-treated T lymphocytes. The JL89 T lymphocytes were preincubated with either normal mouse sera or TS1/22 monoclonal antibody as
25 described above for FIG. 3 and then allowed to adhere to the HeLa cell transfectants for 1 hr at 37°C. PMA was used at 50 ng/ml where indicated.

FIG. 5 is a series of bar graphs showing the effect of neuraminidase on CD43 interference with T-
30 lymphocyte adhesion. JL89 T lymphocytes were preincubated with normal mouse sera or TS1/22 monoclonal antibody as described above for FIG. 3 and then allowed to adhere to untreated or neuraminidase-treated HeLa cell transfectants. *Vibrio cholera* neuraminidase was used at
35 0.02 units per ml.

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There now follows a description of a series of experiments which demonstrates that CD43 protein interferes with intercellular adhesion; these experiments also suggest that CD43 may regulate T-lymphocyte adhesion by interfering with LFA-1-mediated interactions.

Generation and Characterization of Stable HeLa Cell Transfectants Expressing CD43

HeLa cells were chosen to generate stable, CD43-expressing lines because they naturally express ICAM-1 and LFA-3, the counterreceptors for the leukocyte adhesion molecules LFA-1 and CD2, respectively. Toward generating useful cell lines, the PEER-3 cDNA clone encoding CD43 (Pallant, A.S. et al., *Proc. Natl. Acad. Sci. USA*, 86:1328-1332, 1989) was first subcloned into the CDM8 expression vector (Seed, B., *Nature (London)* 329:840-842, 1987) as described in Ardman, B.A. et al. (*J. Exp. Med.*, 172:1151-1158, 1990). HeLa cells (4×10^6) were then cotransfected by electroporation with 2 μ g of the pSV2neo plasmid (encoding neomycin resistance; Southern et al., *J. Mol. Appl. Genet.* 1:327, 1982) and 20 μ g of the CDM8-CD43 plasmid, or transfected alone with pSV2neo. After 72 hr in culture, cells were treated with G418 sulfate at 400 μ g/ml (Geneticin; GIBCO, Grand Island, NY) to select for cells stably transfected by pSV2neo. After 2 weeks in culture, the cotransfected cells were screened by immunofluorescence for CD43 expression, using an anti-CD43 monoclonal antibody, termed anti-Leu 22 (Becton Dickinson, Lincoln Park, NJ). All CD43-positive HeLa cell clones selected by this method consistently expressed CD43 for the duration of the experiments (>6 mo).

The degree of CD43 expression by the positive clones was assessed by flow cytometry and found equivalent to CD43 expression by human T lymphocytes (Fig. 1). The CD43-positive and CD43-negative phenotypes

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of the transfectants were confirmed by anti-Leu 22 immunoprecipitation of solubilized membranes from radiolabeled cells. Specifically, HeLa cell transfectants were surface labeled with Na¹²⁵I (Amersham, 5 Arlington Heights, IL) by using bovine milk lactoperoxidase (Calbiochem-Behring, San Diego, CA). Solubilized cell lysates were then immunoprecipitated as described in Ardman, B.A. et al. (*J. Exp. Med.*, 172:1151-1158, 1990). The immunoprecipitates were resolved by 10% 10 SDS/PAGE under reducing conditions, and the dried gel was autoradiographed at -70°C using an intensifying screen. CD43 protein was observed in the CD43-positive, but not the CD43-negative, HeLa cells. In addition, a separate anti-Leu 22 immunoprecipitate from the CD43-positive HeLa 15 cells was treated with neuraminidase before SDS/PAGE analysis. Following neuraminidase treatment, the CD43 band shifted to a higher M_r, a characteristic feature of CD43.

To determine whether CD43 expression by the 20 transfected HeLa cells was associated with altered expression of ICAM-1 or LFA-3, the CD43-positive HeLa cells were immunophenotyped. Transfectants were stained with either monoclonal antibodies to LFA-1 α subunit (TSI/22), β_2 integrin subunit (TSI/18), ICAM-1 (RR1/1), 25 or LFA-3(TS2/9) (Rothlein, R. et al., *J. Immunol.*, 137:1270-1274, 1986; Sanchez-Madrid, F. et al., *J. Exp. Med.*, 158:1785-1803, 1983; Sanchez-Madrid, F. et al., *Proc. Natl. Acad. Sci. USA*, 79:7489-7493, 1982) at a 1:400 dilution or were stained with purified monoclonal 30 antibody anti-Leu 22 at 5 μ g/ml in an indirect immunofluorescent assay as described in Ardman, B.A. et al. (*J. Exp. Med.*, 172:1151-1158, 1990). The concentration of ascites and purified antibodies was previously determined to contain saturating antibody 35 levels. Normal mouse sera diluted 1:200 served as the

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negative control. Stained cells were analyzed on a Coulter 541 flow cytometer (Coulter).

TABLE 1. Phenotype of the HeLa cell transfectants

5	Antibody	Antigen	Specific linear fluorescence intensity*	
			CD43-negative HeLa cells	CD-43-positive HeLa cells
	RR1/1	ICAM-1	112	109
10	TS2/9	LFA-3	29	22
	anti-Leu 22	CD43	<2	108
	TS1/22	LFA-1 α chain	<2	<2
	TS1/18	β_2 -integrin	<2	<2

15 *Mean fluorescence intensity of cells treated with test antibody after subtraction of background fluorescence with normal mouse serum staining.

The CD43-positive and CD43-negative HeLa cells expressed virtually identical levels of ICAM-1 (Table 1).
 20 LFA-3 expression by the HeLa cell transfectants was substantially less than ICAM-1 expression, and between the transfectants, the CD43-positive cells expressed 25% less LFA-3 than did the CD43-negative cells (Table 1).
 No LFA-1 α or β_2 -integrin chain expression was detected
 25 in either HeLa cell transfectant. Because ICAM-1 expression by the different HeLa cell transfectants was similar, the effect of CD43 on LFA-1-mediated, T-lymphocyte binding to the HeLa cells could be tested.

Effect of CD43 on Lymphocyte Binding to HeLa Cells

30 A heterotypic adhesion assay was used to quantitate binding of lymphoid cells in suspension to adherent, transfected HeLa cells. All assays measuring lymphoid cell binding to the transfected HeLa cells were done in 96-well microtiter plates (Costar, Cambridge,
 35 MA). Wells were seeded with 25×10^3 HeLa cells in complete medium. When the cells were adherent and confluent (24-30 hr later), wells were washed twice with

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RPMI 1640 medium using a multichannel pipettor and then refilled with 50 μ l of complete medium. Lymphoid cells (5×10^6) were washed twice with RPMI 1640 medium, labeled with 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein, acetoxymethyl ester (BCECF; Molecular Probes) in 1 ml of RPMI 1640 medium for 20 min at room temperature, washed, and then resuspended to a concentration of $5-6 \times 10^5$ cells per ml in RPMI medium. Lymphoid cells (100 μ l) were allowed to adhere to the HeLa cells for 30 min at room temperature, and then the wells were washed four times by alternating four-corner vacuum aspiration (25-gauge needle) with gentle addition of 200 μ l of phosphate-buffered saline per well using a multichannel pipettor. The cells were then fixed in 0.37% formalin, and the plates were read on an automated microfluorimeter (Pandex). All assays were done in quadruplicate and repeated three times. Student's *t* test for unpaired samples was used for statistical calculations.

The initial experiments tested the ability of CD43-positive, human T-lymphoid cells from different sources to bind to the CD43-negative and CD43-positive HeLa cells. Cells were obtained from the following sources. Peripheral blood from normal donors was used to isolate mononuclear cells by Ficoll/Hypaque centrifugation. Peripheral blood mononuclear cells were enriched for T lymphocytes by plastic adherence to remove monocytes and nylon fiber filtration to remove B lymphocytes. The resultant T-cell preparations were >90% CD3-positive. T-cell blasts were generated by growing peripheral blood mononuclear cells in phytohemagglutinin at 5 μ g/ml (Sigma) for 3 days and 100 units of recombinant interleukin 2 (IL-2) (Cetus, Norwalk, CT) for 10-17 days. Normal human thymocytes were obtained from infants undergoing corrective cardiac surgery at the New

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England Medical Center. Human lymphocyte cell lines used were SUPT1 (T cell lymphoma), JL89 (IL-2-dependent T-cell clone), and C91/PL (human T-lymphotropic virus type 1-transformed T-cell line). The HeLa cells and the
5 lymphocyte cell lines were grown in RPMI 1640 medium/10% fetal bovine serum (HyClone)/2 mM L-glutamine/penicillin and streptomycin at 100 µg/ml.

All cell types tested, including peripheral blood T lymphocytes, PHA-T cell blasts, thymocytes, two
10 transformed T-cell lines, and an IL-2-dependent T-cell clone bound significantly better to the CD43-negative HeLa Cells than to the CD43-positive HeLa cells (Fig. 2). Similar results were obtained by using two other sets of HeLa cell transfectants derived from different clones
15 (data not shown). These data demonstrate that CD43 expression by the HeLa cell transfectants interfered with T-lymphocyte adhesion. The results also suggest that the anti-adhesion effect of CD43 on T lymphocytes is independent of their maturational stage. Effect of CD43
20 on LFA-1-Mediated Lymphocyte Adhesion

To determine whether CD43 could interfere with LFA-1-mediated lymphocyte adhesion, we measured the proportion of T cells that bound via LFA-1 to each set of HeLa cell transfectants. For these experiments, a human
25 IL-2-dependent T-cell clone (JL89) and PHA-T cells (T cells simulated with PHA for 3 days and maintained in IL-2 for >10 days) were used. Lymphocytes were preincubated for 30 minutes on ice with normal mouse serum or with anti-LFA-1 antibody (TS1/22) to block LFA-1-mediated
30 adhesion (Marlin et al., Cell 51:813-819, 1987) or anti-CD2 (TS2/18) to block CD2-mediated adhesion at a final dilution of 1:200 in RPMI 1640 medium. The lymphocytes then were allowed to adhere to the CD43-positive or CD43-negative HeLa cells. The effect of CD43 on LFA-1-
35 mediated lymphocyte adhesion was determined by comparing

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the percentage of lymphocytes that were blocked from binding to the different HeLa cell transfectants.

Binding of the JL89 lymphocytes was 2.5-fold greater to the CD43-negative than to the CD43-positive HeLa cells (Fig. 3A). Preincubation of the JL89 lymphocytes with the monoclonal antibody TS1-22 resulted in $\approx 80\%$ inhibition of lymphocyte binding to both sets of HeLa cells (Fig. 3A). These data indicate that the substantial majority of the JL89 binding to both sets of HeLa cells was LFA-1-mediated and that the observed interference by CD43 with T-cell binding was largely a result of its interference with LFA-1-mediated adhesion. A similar degree of CD43 interference with LFA-1-mediated adhesion was also observed for PHA-T cell blasts (Fig. 3B), confirming such interference was not secondary to a feature specific to the JL89 T-cell clone. Reciprocal blocking experiments in which the HeLa cell transfectants were preincubated with anti-ICAM-1 ascites (RR1/1) confirmed that CD43 expression by the HeLa cells interfered with LFA-1-mediated T cell binding (data not shown). Preincubation of the lymphocytes with monoclonal antibody to CD2 (TS2-18) did not interfere with T-cell binding in this assay system, a result likely related, in part, to the low expression of LFA-3 by the HeLa cells (see Table 1).

Effect of CD43 on Adhesion of PMA-Activated T Lymphocytes

It has been demonstrated (Rothlein et al., *J. Exp. Med.* 163:1132-1149, 1986) that activation of lymphoid cells by phorbol 12-myristate 13-acetate (PMA) treatment will increase their homotypic adhesion. The mechanism of this increased adhesion results from a PMA-induced increase in the avidity of LFA-1 for ICAM-1 (Dustin et al., *Nature (London)* 341:619-624, 1989). To evaluate whether enhancement of the avidity of LFA-1 for ICAM-1 could overcome CD43 interference of lymphocyte adhesion,

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lymphocytes were allowed to adhere to HeLa cells in the presence of PMA (Sigma, St. Louis, MO) at 50 ng/ml (final concentration) for 60 min at 37°C in a humidified incubator with 5% CO₂. Binding of the JL89 T cell clone
5 to the CD43-negative and CD43-positive HeLa cells was then compared before and after T-lymphocyte activation by PMA. To prevent cell clumping during the coincubation period, 50% of the usual number of T cells (i.e., 25 x 10³ cells) were added to each well.

10 Addition of PMA resulted in increased binding of T cells to both the CD43-negative and CD43-positive transfectants (Fig. 4). However, the increase in the percentage of lymphocytes that bound to the CD43-negative cells was greater than that which bound to the CD43-
15 positive HeLa cells (35% vs. 14%, respectively).

Preincubation of the PMA-treated lymphocytes with the anti-LFA-1 antibody TS1-22 (as described above) resulted in 70-75% inhibition of T-cell binding to both sets of HeLa cell transfectants. Neither the amount of LFA-1
20 expression by lymphocytes nor ICAM-1 expression by the different HeLa cell transfectants changed after PMA treatment (data not shown), eliminating the possibility that quantitative differences in receptor expression accounted for the binding differences. These data
25 indicate that the anti-adhesion effect of CD43 was not overcome by PMA treatment of the T lymphocytes. The results also suggest that CD43 can interfere with LFA-1-mediated T-lymphocyte adhesion, irrespective of the avidity of LFA-1 for ICAM-1.

30 Effect of Neuraminidase on CD43 Interference with LFA-1-Mediated Adhesion

A notable feature of CD43 is its extensive substitution with negatively charged, sialic acid residues (Remold et al., *J. Biol. Chem.* 261:7526, 1986;
35 Carlsson et al., *J. Biol. Chem.* 261:12779, 1986). It has

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been proposed that these sialic acid residues confer an anti-adhesion function to CD43 by providing a net negative charge to cell surfaces, resulting in repulsion between CD43-positive cells (Brown et al., *Nature* 5 (London) 289:456-460, 1981). To determine whether sialic acid residues of CD43 contribute to its interference with cell-cell adhesion, the HeLa cell transfectants were treated with neuraminidase before testing for T-lymphocyte adhesion. HeLa cells were incubated with 10 *Vibrio cholerae* neuraminidase (0.02 unit per ml; Calbiochem-Behring) in RPMI 1640 medium for 1 hr at 37°C followed by extensive washing of cells. This concentration of neuraminidase (0.02 unit per ml) was predetermined to completely eliminate the sialic acid- 15 dependent epitope recognized by anti-Leu 22, while not affecting cell adherence to the polystyrene wells.

Neuraminidase treatment of the HeLa cells increased T-cell adhesion to both the CD43-negative and CD43-positive transfectants (Fig. 5). However, the 20 increase in T-lymphocyte binding to the CD43-positive HeLa cells was significantly greater than the increase in lymphocyte binding to the CD43-negative cells (18.5% vs. 8.8%, respectively; see Fig. 5). Preincubation of the neuraminidase-treated lymphocytes with the anti-LFA-1 25 antibody TS1/22 resulted in 80% inhibition of T-cell binding to both sets of HeLa cell transfectants. These results suggested that the removal of sialic acid residues from CD43 was responsible for the disproportionate increase in T-cell adhesion to the CD43- 30 positive HeLa cells and that this increased adhesion was LFA-1-mediated. It should be noted, however, that despite neuraminidase treatment, the total percentage of T lymphocytes binding via LFA-1 to the CD43-positive HeLa cells remained significantly less than that to the CD43- 35 negative cells (32.7 vs. 46.0%, respectively; Fig. 5).

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Taken together, these data suggest that the sialic acid residues of CD43 contribute to, but are not wholly responsible for, its ability to interfere with LFA-1-mediated T-lymphocyte adhesion.

5 CD43 Acts to Block Cellular Interactions

The results described above demonstrate that CD43 (leukosialin or sialophorin), when expressed by opposing cells, functions as an anti-adhesion molecule. CD43 expression by HeLa cells interfered significantly with
10 adhesion of T lymphocytes, cells that naturally express CD43. The interference occurred at physiologic levels of CD43 expression by the HeLa cell transfectants and was observed for T lymphocytes, irrespective of their source or derivation. The results also show that CD43
15 expression by opposing cells interferes with LFA-1-mediated T-cell adhesion. Under the assay conditions used, most T-cell adhesion was LFA-1-mediated (see Fig. 3). Consequently, the observed interference by CD43 with T-cell binding represented interference with LFA-1-
20 mediated adhesion. Because LFA-1-mediated adhesion constitutes a dominant adhesion pathway among lymphocytes (Mentzer et al., *J. Immunol.* 135:9-11, 1985; Rothlein et al., *J. Exp. Med.* 163:1132-1149, 1986), the results described herein suggest that CD43 may regulate adhesion
25 among T lymphocytes by interfering with LFA-1-mediated adhesion.

LFA-1 is one of several activation-dependent intercellular adhesion molecules and PMA treatment of LFA-1-positive cells substantially enhances their binding
30 avidity to ICAM-1 (Dustin et al., *Nature* (London) 341:619-624, 1989). The results described above are consistent with these data, as PMA treatment substantially increased the percentage of T-cell binding to the CD43-negative, ICAM-1-positive HeLa cells.
35 However, the PMA-induced increase in T-cell binding to

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the CD43-positive HeLa cells was blunted in comparison to that for the CD43-negative HeLa cells (see Fig. 4).

These results suggest that the anti-adhesion effect of CD43 is not overcome by enhancement of the avidity of

5 LFA-1 for ICAM-1.

Two distinctive features of CD43 are the extensive O-glycosylation and sialylation of its extracellular domain (Remod-O'Donnell et al., *J. Biol. Chem.* 261:7526-7530, 1986; Carlsson et al., *J. Biol. Chem.* 261:12779-10 12786, 1986; Pallant et al., *Proc. Natl. Acad. Sci. USA* 86:1328-1332, 1989; Shelly et al., *Proc. Natl. Acad. Sci. USA* 86:2819-2823, 1989). The sialic acid residues impart a negative charge to CD43 and could limit the association of CD43-positive cells with each other by repulsion of 15 like charge. The results of the neuraminidase-treatment experiments in the present study suggest that sialic acid residues of CD43 contribute to, but are not completely responsible for, its anti-adhesion effect (see Fig. 5). Because the efficacy of neuraminidase treatment was 20 gauged by the ability to eliminate a CD43 sialic acid-dependent epitope (anti-Leu 22 reactive) from the CD43-positive HeLa cells, it is possible that other sialic acid residues on CD43 remained intact. Under these circumstances, the sialic acid contribution to the CD43 25 anti-adhesion effect may have been underestimated. However, in other experiments where HeLa cell transfectants were treated with a concentration of neuraminidase 5-fold greater than that used above (0.1 unit per ml), no further specific increase in T-cell 30 binding was noted. These observations suggest that it is likely that sialic acid is not completely responsible for the CD43 anti-adhesion effect.

The large number of O-linked carbohydrates spanning the entire extracellular domain of CD43 (one 35 potential O-linked site per three amino acids, on

- 20 -

average) (Pallant et al., *Proc. Natl. Acad. Sci. USA* 86:1328-1332, 1989; Southern et al., *J. Mol. Appl. Genet.* 1:327-341, 1982) suggests that CD43 exists as an unfolded structure extending from the cell surface (Jentoft, 5 *Trends Biochem. Sci.* 15:291-294, 1990). Recently, the extracellular domain of rat CD43 has been measured to extend 45 nm (Cyster et al., *EMBO J.* 10:893-902, 1991). These structural features of CD43 are consistent with its ability to interfere with LFA-1-mediated T-cell binding, 10 an interaction predicted to occur within an intermembrane distance of 25-30 nm (Springer, *Nature (London)* 346:425-434, 1990). Other receptor-counterreceptor pairs (e.g., CD2/LFA-3) are predicted to require intermembrane distances even smaller than that for LFA-1/ICAM-1, 15 suggesting that their ability to interact would be diminished substantially by CD43 expression. Although the experiments described herein were designed primarily to detect the effect of CD43 on LFA-1-mediated T-cell adhesion, in other experiments the observation has been 20 made that the CD43-positive HeLa cells, in suspension, fail to aggregate, whereas the CD43-negative HeLa cells readily form aggregates (data not shown). Because HeLa cells do not express LFA-1, the inability of the CD43-positive HeLa cells to aggregate supports the notion that 25 CD43 interference with cell-cell adhesion is not specifically limited to interference with LFA-1-mediated binding.

Others have shown that CD43 has pro-adhesive features (Park et al., *Nature (London)* 350:706-709, 1991) 30 and can bind directly to ICAM-1 (Rosenstein et al., *Nature (London)* 354:233-235, 1991). In the latter study, a Burkitt lymphoma-derived B-cell line, Daudi (CD43-negative), was shown to bind to CD43, and a murine hybridoma expressing human CD43 was shown to bind to 35 ICAM-1. Although there is as yet no demonstration that

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CD43-positive HeLa cells bind to purified soluble CD43, such conflicting results might indicate that under different conditions, CD43 can have both pro-adhesive and anti-adhesive activities, similar to that seen for the
5 heavily glycosylated, neural cell adhesion molecule NCAM (Rutishauser et al., *Science* 240:53-57, 1988). The conditions of the adhesion assay used in the above study were designed to detect the effect of CD43 expression by opposing cells, in an attempt to mimic interactions among
10 CD43-positive lymphocytes.

The threshold for intercellular adhesion might depend, to a large degree, upon whether CD43 is expressed by one or both interacting cells. For example, the binding threshold between CD43-positive lymphocytes might
15 be expected to be greater than that between lymphocytes and CD43-negative cells (e.g., high endothelial venules). It is also possible that the *in vivo* effect(s) of CD43 depend upon the extent of its sialylation in addition to its tissue distribution. Incompletely sialylated CD43, a
20 form naturally expressed by thymocytes (Ardman et al., *J. Exp. Med.* 172:1151-1158, 1990; Remold-O'Donnell et al., *Blood* 70:104-109, 1987; Borche et al., *Eur. J. Immunol.* 17:1523-1526, 1987) and, perhaps, by activated germinal-center B lymphocytes (Butcher et al., *J. Immunol.*
25 129:2698-2707, 1986), exposes a core structure rich in oligosaccharides that may promote cell adherence to adjacent cells or stroma. In contrast, the fully sialylated form that is expressed by mature T lymphocytes (Remold-O'Donnell et al., *Blood* 70:104-109, 1987; Borche
30 et al., *Eur. J. Immunol.* 17:1523-1526, 1987) could function to limit adhesion between CD43-positive cells.

Construction of CD43 Chimeras

Because the CD43 protein blocks cellular interactions, this protein can be used generally to coat
35 a target cell surface and prevent deleterious

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intercellular interactions, for example, those cellular interactions leading to inflammation. To direct the CD43 protein to the proper target cell surface, a CD43 chimeric molecule is constructed which includes (a) a cell targeting molecule which directs specific binding of the chimeric molecule to a molecule (e.g., a receptor, ligand, or counterreceptor) on the surface of one or both of the cells whose interaction is to be disrupted and (b) a CD43 extracellular domain bearing a net negative charge at physiological pH which antagonizes the intercellular interaction. Such a chimeric protein should be soluble to facilitate administration.

Two particularly preferred chimeric proteins are CD43:LNF III and CD43:sialyl Lewis^x. LNF III and sialyl Lewis^x (both molecules on the surface of leukocyte cells) interact specifically with P-selectin and ELAM-1 (respectively), receptors present on the surface of endothelial cells activated during inflammation; such immune cell-endothelial cell interactions are required for progression of the inflammatory response. Accordingly, therapeutic CD43:LNF III or CD43:sialyl Lewis^x chimeric molecules can be administered in order to coat the endothelial cell surface with CD43 protein (specifically blocking interaction with immune cells) and thereby treat or prevent inflammation.

In another approach to treat inflammation, a negatively charged CD43 extracellular domain is fused to the receptors VCAM-1 and ICAM-1 to produce chimeric proteins CD43:VCAM-1 and CD43:ICAM-1; such proteins coat the surface of certain types of leukocytes, and again block the intracellular interaction between the immune cells and the endothelium which leads to inflammation.

To construct CD43:LNF III, CD43:sialyl Lewis^x, CD43:ICAM-1, CD43:VCAM-1, or any other CD43 chimera, an extracellular (soluble) portion of the CD43 protein

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bearing a net negative charge at physiological pH is covalently bonded to the cell targeting molecule of choice. Preferably, the CD43 domain is not only negatively charged but also of sufficient size to

5 physically interfere with the intercellular interaction. Preferably, the CD43 domain includes the N-terminal 180 amino acids and, more preferably, includes the N-terminal 220-233 amino acids of the CD43 sequence (as described, e.g., in Shelley et al., *Proc. Natl. Acad. Sci. USA*

10 86:2819, 1989; Pallant et al., *Proc. Natl. Acad. Sci. USA* 86:1328, 1989).

The cell targeting molecule can be chosen from ligands, receptors, counterreceptors, antibodies, carbohydrates, or any molecule which is capable of

15 specific interaction with a surface molecule on the targeted cell. The cell targeting domain must also be soluble and, in addition, must be of sufficient size and composition to direct specific interaction with its binding partner on the target cell surface. For ICAM-1,

20 the cell targeting domain preferably includes between the N-terminal 430 and N-terminal 452 amino acids of the ICAM-1 sequence described by Staunton et al. (*Cell* 52:925, 1988); for VCAM-1, the cell targeting domain preferably includes between the N-terminal 580 and the N-

25 terminal 606 amino acids of the VCAM-1 sequence described by Osborn et al. (*Cell* 59:1209, 1989); and for CD4, the cell targeting domain preferably includes between the N-terminal 350 and the N-terminal 371 amino acids of the CD4 sequence described by Maddon et al. (*Cell* 42:93,

30 1986) and Hussey et al. (*Nature* 331:78, 1988).

The relative positions of the CD43 and the cell targeting domains within the chimeric protein are not critical to the chimeric molecule's function, although the orientation, N-terminus--Cell Targeting Molecule--

35 CD43--C-terminus is preferred. To increase the

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flexibility of the domains and thereby increase the ease with which each domain may interact with other molecules (e.g., opposing CD43 molecules or cell surface ligands, receptors, or counterreceptors), a proline-rich hinge
5 region of variable length may be inserted between the two domains.

The chimeric molecules of the invention are preferably chimeric proteins expressed from chimeric fusion genes designed and constructed using standard
10 techniques of molecular biology (see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Press, Cold Spring Harbor, NY; or Ausubel et al., *Current Protocols in Molecular Biology*, Wiley Interscience, 1989). Chimeric molecules may also be
15 produced, however, by chemical coupling of the proteins, protein fragments, or molecules of the chimera. The chemical coupling approach is the method of choice for CD43:carbohydrate chimeras such as CD43:LNF III and CD43:sialyl Lewis^x.

20 Therapy

The CD43 chimeric molecules of the invention are generally useful for blocking deleterious intercellular interactions, and, in particular, are useful for antagonizing the endothelial cell-immune cell
25 interactions leading to inflammation and host cell-pathogen cell interactions leading to pathogen infectivity.

For administration, therapeutic CD43 chimeric molecules are formulated in an appropriate
30 pharmaceutically-acceptable buffer such as physiological saline. The therapeutic preparation is administered in accordance with the condition to be treated. Ordinarily, it will be administered intravenously, at a dosage that provides suitable competition for the deleterious
35 intercellular interaction; such a dosage will normally be

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in the range of 0.01 to 100 mg/kg/day, preferably 0.1 to 5 mg/kg/day. Alternatively, it may be convenient to administer the therapeutic orally, nasally, parenterally, subcutaneously, or topically, e.g., as an ointment, a liquid or a spray. For rheumatoid arthritis, the therapeutic preparation may be injected directly into the inflamed joint. Again, the dosages are as described above. Treatment may be repeated as necessary for alleviation or prevention of disease symptoms.

10 The CD43:LNF III, CD43:sialyl Lewis^x, CD43:ICAM-1, and CD43:VCAM-1 therapeutic proteins are particularly useful for blocking interactions between white blood cells and activated endothelium and are therefore useful for treating or preventing inflammatory disorders, for
15 example, the vasculitis disorders, lupus and rheumatoid arthritis, as well as for suppressing downstream immune responses leading, for example, to organ rejection. CD43:ICAM-1 may also be used to disrupt cell-mediated, organ-specific inflammation secondary to virus infection
20 thus could be useful for treating disorders such as viral myocarditis.

 The methods and therapeutics described herein may be used to treat disorders in any mammal, for example, humans, domestic pets, or livestock. Where a non-human
25 mammal is treated, the CD43 or cell targeting proteins employed in construction of the therapeutic CD43 chimeric protein are preferably specific for that species.

 Other embodiments are within the following claims.

 What is claimed is:

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Claims

1. Use of a soluble chimeric molecule comprising (a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of said first cell covalently bonded to (b) a CD43 extracellular domain bearing a net negative charge at physiological pH to inhibit an interaction between a first and a second cell for treatment of a disease in a mammal.
- 10 2. The use of claim 1, wherein said second cell bears a negatively-charged molecule on its cell surface.
3. The use of claim 2, wherein said negatively-charged cell surface molecule is CD43.
4. The use of claim 1, wherein said CD43
15 extracellular domain is glycosylated.
5. The use of claim 1, wherein said CD43 extracellular domain comprises the N-terminal 180 amino acids of the CD43 protein sequence.
6. The use of claim 1, wherein said cell
20 targeting molecule is a P-selectin-binding portion of an LNF III receptor protein.
7. The use of claim 1, wherein said cell targeting molecule is an ELAM-1-binding portion of a sialyl Lewis^x receptor protein.
- 25 8. The use of claim 1, wherein said cell targeting molecule is an LFA-1-binding portion of an ICAM-1 receptor protein.

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9. The use of claim 1, wherein said cell targeting molecule is a VLA-4-binding portion of a VCAM-1 receptor protein.

10. The use of claim 1, wherein said cell targeting molecule is a human immunodeficiency virus-binding portion of CD4.

11. The use of claim 1, wherein said cell targeting molecule is a rhinovirus-binding portion of ICAM-1.

12. The use of claim 1, wherein said cell targeting molecule is a Plasmodium-binding portion of ICAM-1.

13. The use of claim 1, wherein said first cell is an activated endothelial cell.

14. The use of claim 1, wherein said first cell is an infective pathogen.

15. The use of claim 14, wherein said infective pathogen is rhinovirus, *Plasmodium falciparum*, *Plasmodium malariae*, or a human immunodeficiency virus.

16. A chimeric molecule, comprising (a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of a cell joined to (b) a CD43 extracellular domain bearing a net negative charge at physiological pH.

17. The chimeric molecule of claim 16, wherein said CD43 extracellular domain is glycosylated.

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18. The chimeric molecule of claim 16, wherein said CD43 extracellular domain comprises the N-terminal 180 amino acids of the CD43 protein sequence.

19. The chimeric molecule of claim 16, wherein
5 said cell targeting molecule is a P-selectin-binding portion of an LNF III receptor protein.

20. The chimeric molecule of claim 16, wherein said cell targeting molecule is an ELAM-1-binding portion of a sialyl Lewis^x receptor protein.

10 21. The chimeric molecule of claim 16, wherein said cell targeting molecule is an LFA-1-binding portion of an ICAM-1 receptor protein.

22. The chimeric molecule of claim 16, wherein said cell targeting molecule is a VLA-4-binding portion
15 of a VCAM-1 receptor protein.

23. The chimeric molecule of claim 16, wherein said cell targeting molecule is a human immunodeficiency virus-binding portion of CD4.

24. The chimeric molecule of claim 16, wherein
20 said cell targeting molecule is a rhinovirus-binding portion of ICAM-1.

25. The chimeric molecule of claim 16, wherein said cell targeting molecule is a Plasmodium-binding portion of ICAM-1.

25 26. The chimeric molecule of claim 16, wherein said cell targeting molecule specifically recognizes and

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binds to a molecule on the surface of an activated endothelial cell.

27. The chimeric molecule of claim 16, wherein said cell targeting molecule specifically recognizes and
5 binds to an infective pathogen.

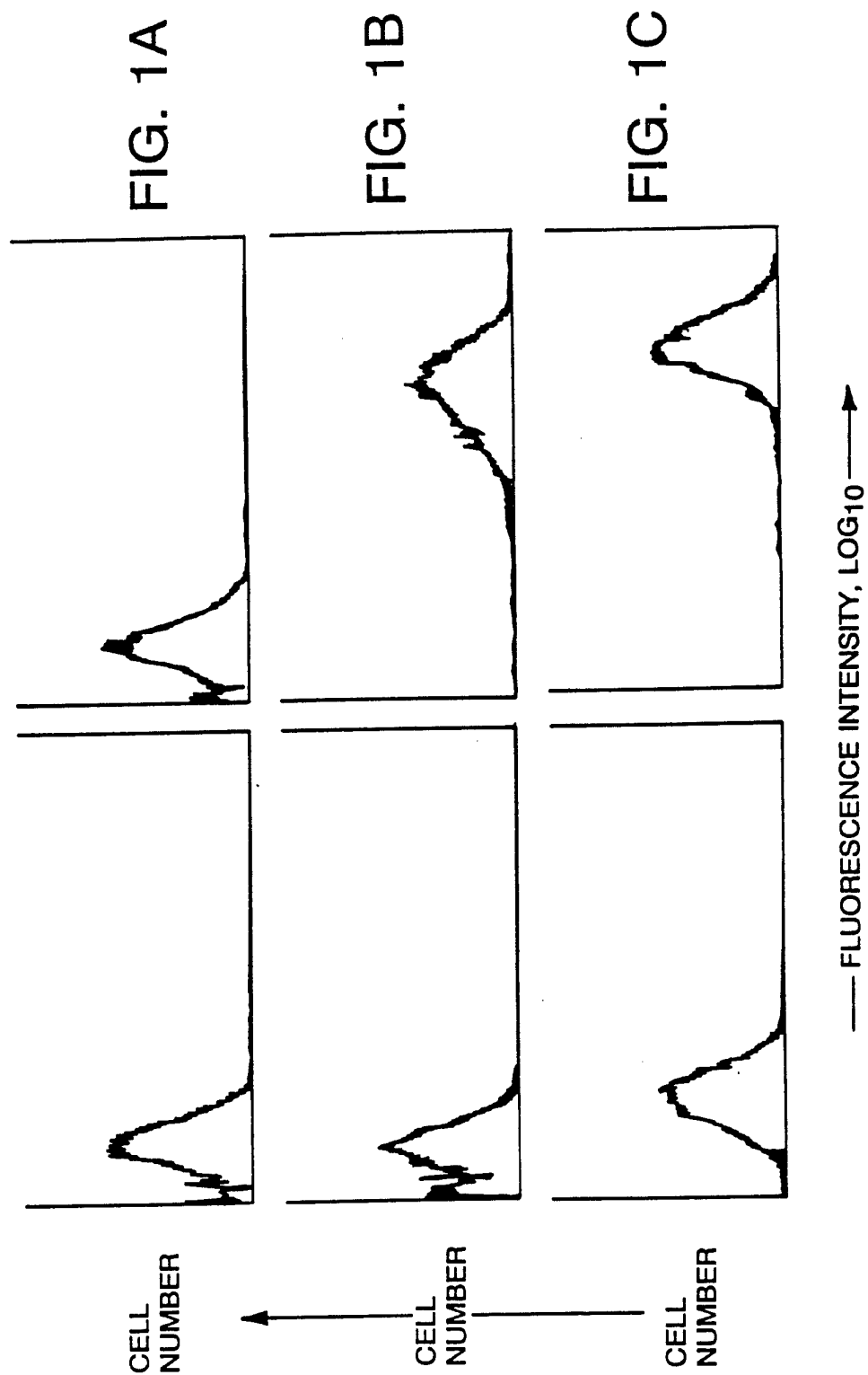
28. Use of a chimeric molecule comprising (a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of an activated endothelial cell joined to (b) a CD43
10 extracellular domain bearing a net negative charge at physiological pH to inhibit intercellular interaction between a said activated endothelial cell and white blood cells for the treatment of inflammation in a mammal.

29. The use of claim 28, wherein said cell
15 targeting molecule is a P-selectin-binding portion of an LNF III receptor protein.

30. The use of claim 28, wherein said cell targeting molecule is an ELAM-1-binding portion of a sialyl Lewis^x receptor protein.

20 31. Use of a chimeric molecule comprising (a) a cell targeting molecule which is capable of specifically recognizing and binding to a molecule on the surface of a pathogen joined to (b) a CD43 extracellular domain bearing a net negative charge at physiological pH to
25 inhibit intercellular interaction between said pathogen and the mammalian cell it normally infects to treat an infection of a mammal with said pathogen.

32. The use of claim 31, wherein said pathogen is a human immunodeficiency virus.



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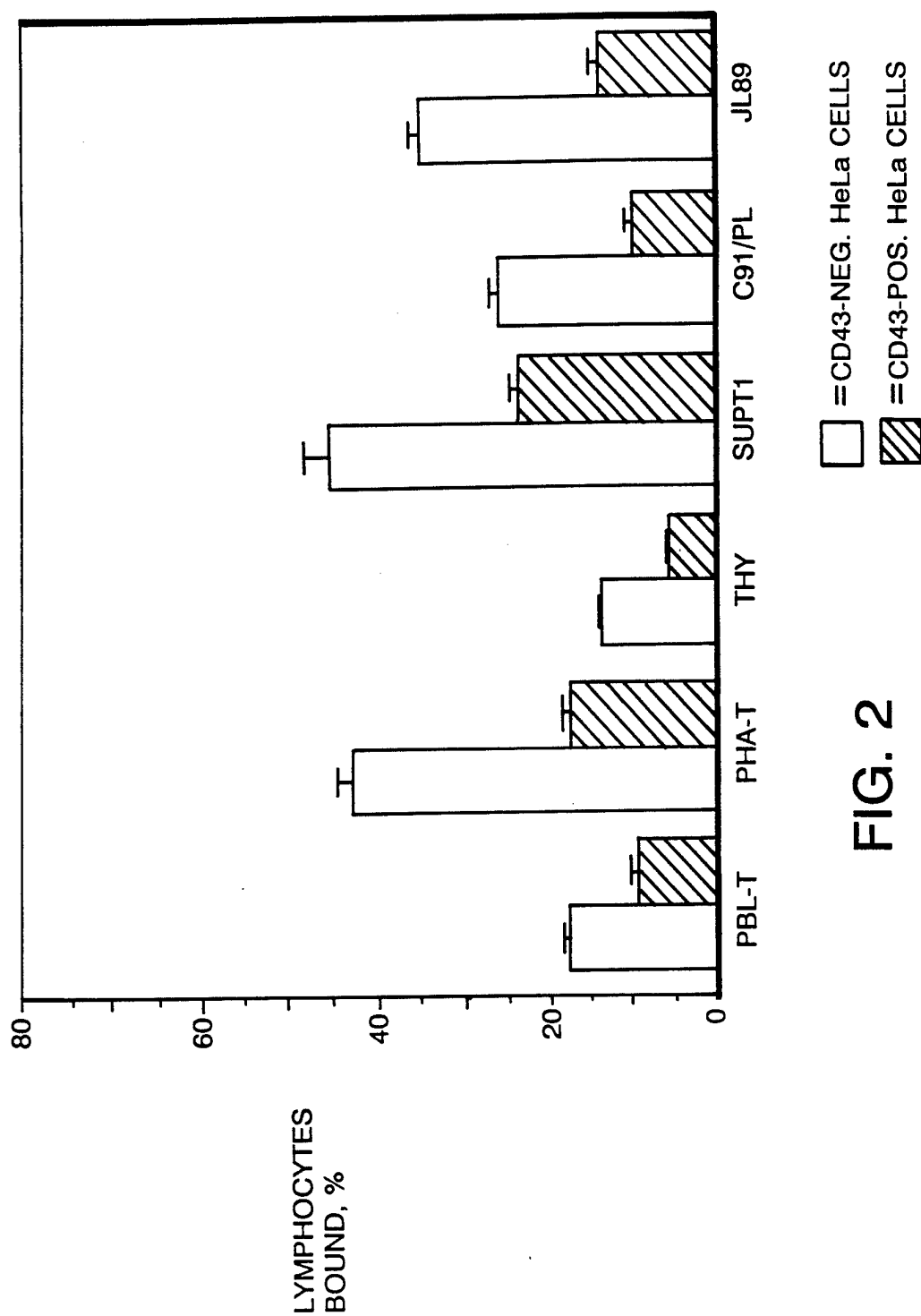


FIG. 2

SUBSTITUTE SHEET

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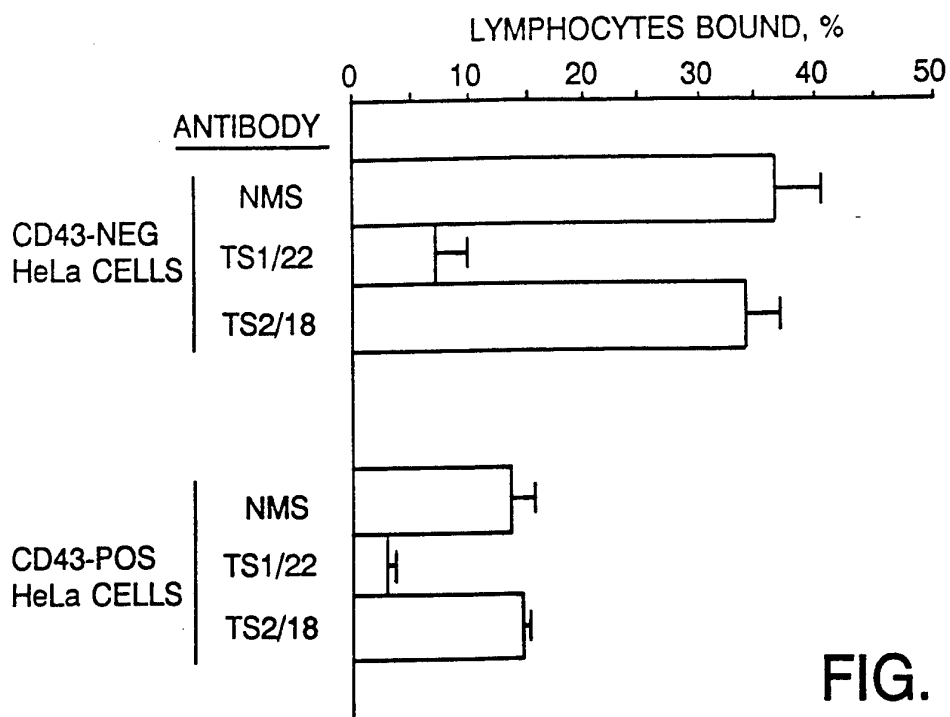


FIG. 3A

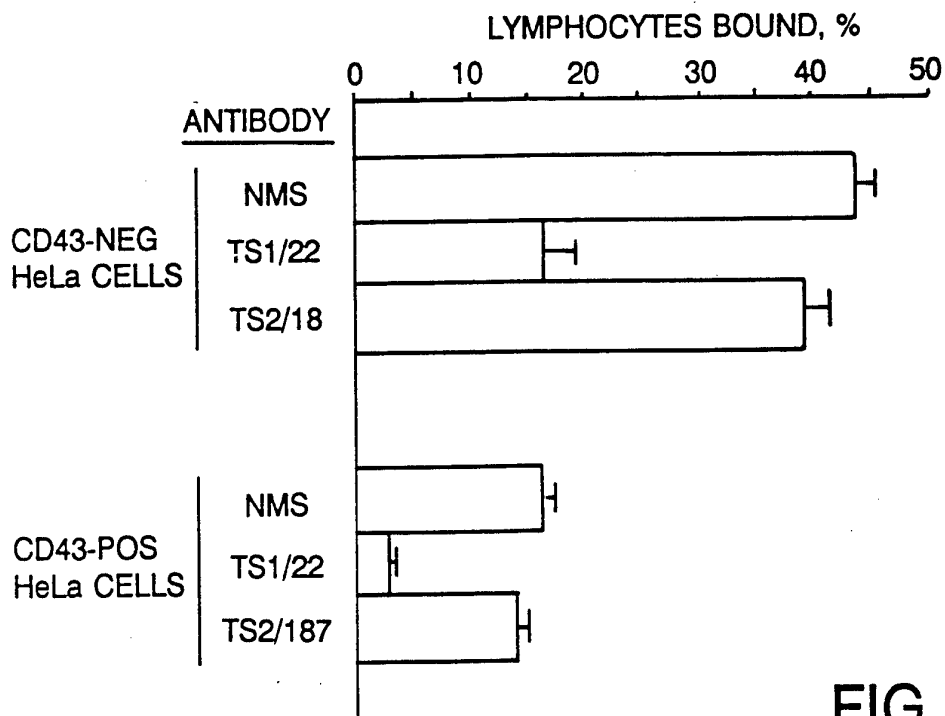


FIG. 3B

SUBSTITUTE SHEET

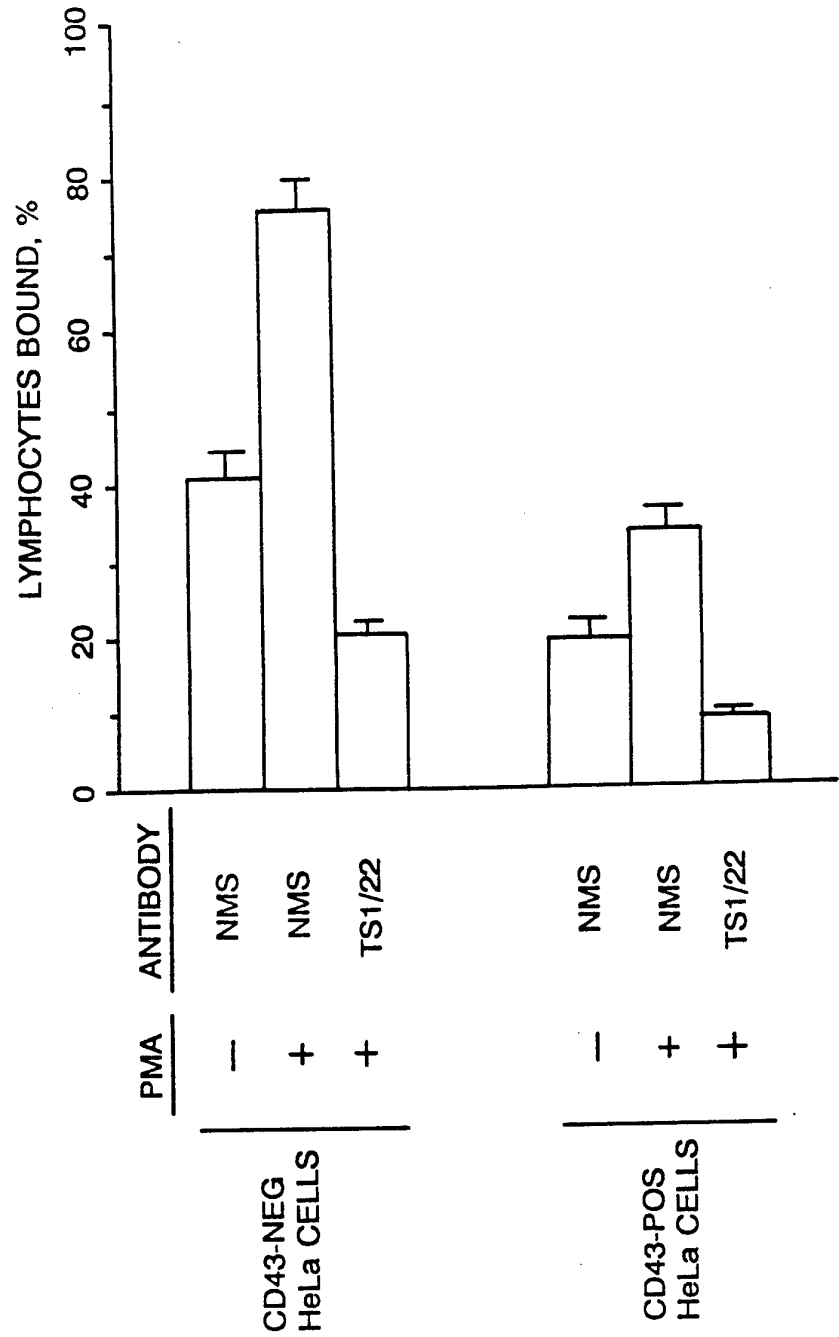


FIG. 4

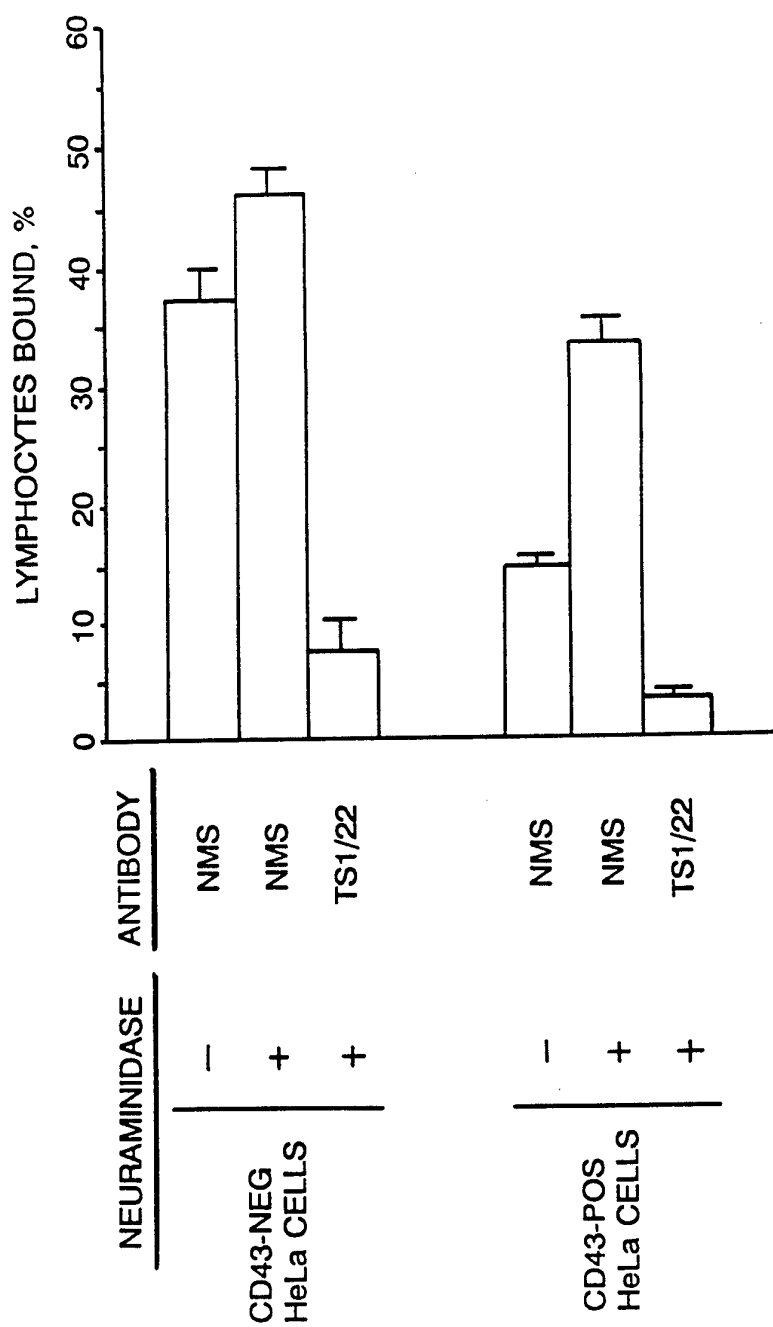


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04517**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : A61K 37/02; C12P 21/06; C12N 15/00; C07K 13/00

US CL : 514/8; 435/69.7, 172.3; 530/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/8; 435/69.7, 172.3; 530/395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG

search terms: CD43, selectin, elam, LFA, ICAM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EMBO Journal, Volume 10(4), issued 1991, J.G. Cyster et al., "The dimensions of the T lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation", pages 893-902, entire document.	1-32
A	Science, Volume 254, issued 22 NOVEMBER 1991, I. Pastan and D. FitzGerald, "Recombinant Toxins for Cancer Treatment", pages 1173-1177, entire document.	1-32
A	Nature, Volume 354, issued 21 NOVEMBER 1991, Y. Rosenstein et al., "CD43, a molecule defective in Wiskott-Aldrich syndrome, binds ICAM-1", pages 233-235, entire document.	1-32



Further documents are listed in the continuation of Box C.



See patent family annex.

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A

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

E

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L

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

P

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

T

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

X

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y

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

Z

document member of the same patent family

Date of the actual completion of the international search

30 June 1993

Date of mailing of the international search report

03 AUG 1993

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Authorized officer

LORRAINE M. SPECTOR, PH.D.

Telephone No. (703) 308-0196