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(54) **Title:** METHODS AND COMPOSITIONS FOR THE KILLING OF CELLS EXPRESSING HUMAN CD59

(57) **Abstract:** This invention features compositions and methods including intermedilysin for the treatment of conditions associated with human CD59 expression. The invention also features methods and compositions including intermedilysin for the prevention of pregnancy. Additionally, the invention features methods for killing cells expressing human CD59 in a non-human animal.

METHODS AND COMPOSITIONS FOR THE KILLING OF CELLS EXPRESSING HUMAN CD59

Field of the invention

This invention relates to the treatment of obesity and cancer, and the prevention of pregnancy.

Background of the Invention

Throughout the world, the prevalence of obesity is on the increase. There are over 300 million obese adults (Body Mass Index (BMI)>30), according to the World Health Organization, and 1.1 billion overweight people (BMI>25) worldwide. In the United States, more than half of adults are overweight (64.5 percent) and nearly one-third (30.5 percent) are obese. Obesity is associated with conditions such as type 2 diabetes, coronary artery disease, increased incidence of certain cancers, respiratory complications, and osteoarthritis. Being overweight or obese are well-recognized factors that reduce life expectancy and are estimated to cause 300,000 premature deaths each year in the U.S. Medical guidelines to treat obese patients advise changes in eating habits and increased physical activity. Some therapeutic agents exist to aid in the treatment of obesity, however, they cannot substitute for changes in lifestyle.

Intermedilysin (ILY) is a cholesterol-dependent cytolysin secreted by *Streptococcus intermedius* (SI), long suspected to play an important role in the pathogenesis of infectious disease. SI, a gram-positive bacteria, can cause purulent infections in the mouth and internal organs, specifically in the brain and liver. Infections with SI in the brain and liver can lead to abscesses. ILY was assigned to the cholesterol-dependent cytolysin family and shows specific hemolytic activity towards only human erythrocytes, but not towards other animal erythrocytes.

Summary of the Invention

In one aspect, this invention features compositions containing substantially pure ILY (e.g., recombinant ILY) formulated (e.g., a pharmaceutical formulation) for contraceptive or topical administration.

In another aspect, the invention features a method of treating obesity or an obesity related syndrome, a proliferative disease (e.g., melanoma), or a skin-related

disorder in a subject by administering (e.g., locally administering) substantially pure ILY (e.g., recombinant ILY) to the subject. The proliferative disease may be characterized by, for example, neoplastic cells expressing CD59.

In yet another aspect, the invention features a method for preventing pregnancy in a mammal (e.g., a human), by administering into the reproductive tract of the mammal an effective contraceptive amount of substantially pure ILY. The substantially pure ILY may be administered, for example, by topical administration to the female genitalia of the mammal prior to sexual intercourse. The substantially pure ILY may be administered in combination with a pharmaceutically effective excipient, carrier, or diluent, to form a cream, lotion, gel, spray, ointment, paste, jelly, or foam. The substantially pure ILY may also be administered by applying the substantially pure ILY on a male condom, a female condom, a contraceptive diaphragm, a tampon, a pessary, or a contraceptive sponge, prior to sexual intercourse. In addition, the substantially pure ILY may administered in the form of a contraceptive membrane suppository.

The invention also features a method of killing a target cell by expressing human CD59 in the target cell, administering intermedilysin to the target cell thereby killing the target cell. Target cells may be in a non-human animal, for example, in a mouse, rat, sheep, primate, dog, cat, guinea pig, cow, horse, or hamster. In this aspect, the non-human animal may be transgenic animal including a human CD59 gene.

In a related aspect, the invention features a transgenic non-human animal including a gene encoding human CD59.

In any of the above aspects, the transgenic animal may further include an exogenous recombinase gene (e.g., a Cre recombinase or FLP recombinase gene). Conditional expression (e.g., tissue specific or cell type specific expression) of the recombinase protein results in expression of the human CD59 protein.

In any of the above aspects, the human CD59 gene and/or recombinase gene can be conditionally expressed, such that, for example, the gene is only expressed in a certain cell type (e.g., a certain cell type at a certain developmental stage) or tissue (e.g., neural tissue, hematopoietic tissue, skin, endothelial tissue, and muscular tissue).

By "substantially pure" is meant a preparation that is at least 50% by weight (dry weight) ILY. The term "composition comprising substantially pure ILY" is meant to include any ratio of substantially pure intermedilysin to other compounds.

By "obesity" is meant a condition in a human characterized by excessive weight due to stored fat. According to established standards, humans are "overweight" when they have a Body Mass Index (BMI) of greater than 25 and they are "obese" then they have a BMI of greater than 30.

By "obesity related syndromes" is meant conditions including but not limited to depression, type 2 diabetes, dyslipidemia, respiratory complications, sleep apnea, hypertension, gall bladder disease, heart disease (e.g., coronary artery disease), and ostheoarthritis. This term is also meant to include any occurrence of undesirable adipose tissue.

By "contraceptive" and "contraception" are meant compounds useful for preventing full-term pregnancy.

By "subject" is meant a human or other mammal expressing a form of CD59 that sensitizes the expressing cell to ILY.

By "intermedilysin" or "ILY" is meant a polypeptide having the activity of a *Streptococcus intermedius* ILY polypeptide. ILY can be purified from *Streptococcus intermedius*, or can be produced recombinantly. An exemplary Genbank Accession number corresponding to the nucleic acid sequence of ILY is AB029317 and an exemplary Genbank Accession number corresponding to a polypeptide sequence of ILY is BAE16324. An ILY polypeptide can have at least 50%, 60%, 70%, 80%, 90%, 95%, or 99%, sequence identity to the ILY polypeptide having the sequence of BAE16324, or a fragment having ILY activity (e.g., a fragment of the polypeptide having the sequence of BAE16324). Additionally, an ILY polypeptide can be encoded by a nucleic acid that hybridizes under high stringency conditions to a nucleic acid of ILY. ILY can be isolated from any *Streptococcus intermedius* strain (e.g., strains 1208-1, UNS35, UNS46, and ATCC27335), or produced recombinantly.

The "percent sequence identity" of two nucleic acid or polypeptide sequences can be readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, Academic Press, 1987; and

Sequence Analysis Primer, Gribskov, and Devereux, eds., M. Stockton Press, New York, 1991; and Carillo and Lipman, SIAM J. Applied Math. 48:1073, 1988.

Methods to determine identity are available in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package (Devereux et al., Nucleic Acids Research 12:387, 1984), BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol. 215:403, 1990). The well known Smith Waterman algorithm may also be used to determine identity. The BLAST program is publicly available from NCBI and other sources (BLAST Manual, Altschul, et al., NCBI NLM NIH Bethesda, Md. 20894). Searches can be performed in URLs such as: http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi?. These software programs match similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "hybridize" is meant to form a double-stranded complex containing complementary paired nucleobase sequences, or portions thereof, under various conditions of stringency (see, e.g., Wahl. and Berger, Methods Enzymol. 152:399 (1987); Kimmel, Methods Enzymol. 152:507 (1987)).

By "hybridizes under high stringency conditions" is meant under conditions of stringent salt concentration, stringent temperature, or in the presence of formamide. For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the

art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and most preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180 (1977)); Grunstein and Hogness (Proc. Natl. Acad. Sci. USA 72:3961 (1975)); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York (2001)); Berger and Kimmel (Guide to Molecular Cloning Techniques, Academic Press, New York, (1987)); and Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York). Preferably, hybridization occurs under physiological conditions. Typically, complementary nucleobases hybridize via hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding,

between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "proliferative disease" is meant a disease characterized by abnormal cell growth, abnormal lack of cellular apoptosis, or an abnormal decrease in cellular differentiation.

By "having the activity of a Streptococcus intermedius ILY polypeptide" is meant a cholesterol-dependent cytolysing activity specific to cells expressing human CD59.

By "human CD59" or "hCD59" is meant a protein encoded by a nucleic acid sequence substantially similar to SEQ ID NO:1, or functional fragment thereof. An hCD59 protein, or functional fragment thereof, when expressed on the surface of a cell, permits the cytolytic activity of Streptococcus intermedius intermedilysin.polypeptide. By hCD59 is also meant a polypeptide with at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% percent sequence identity to the protein encoded by the cDNA sequence of SEQ ID NO:1. Additionally and alternatively, hCD59 is defined as a polypeptide encoded by a nucleic acid that hybridizes under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1.

Brief Description of the Drawings

Figure 1A is a graph showing percent lysis of red blood cells (RBCs) when treated with ILY. The RBCs of *hCD59RBC*$^{+/-}$ mice, but not those of wild-type (WT) mice are hyper-sensitive to *ex vivo* ILY mediated lysis, at a level comparable to that of human RBC.

Figure 1B is a graph showing the induction of hemolysis in *hCD59RBC*$^{+/-}$ resulting from an ILY (45 ng ILY/g body weight) tail vein injection

Figure 1C is a photograph showing visible hemolysis in samples collected from five *hCD59RBC*$^{+/-}$ mice, but not in those samples from five WT mice. Samples were processed in hematocrit tubes obtained from mouse vein tail 10 minutes after ILY injection (45 ng/g body weight).

Figure 1D is a photograph showing hemoglobinuria in *hCD59RBC*$^{+/-}$ mice but not in WT mice at 5 hours and 1 day after ILY administration (45 ng ILY/g body weight).

Figure 2A is a graph showing percent ILY mediated hemolysis. To evaluate the protective effect of human serum against ILY-mediated hemolysis of serum, serial dilutions of ILY were added to human RBC incubated with a 1 to 8 dilution of serum from different species.

Figure 2B is a graph showing percent ILY mediated hemolysis as a functional comparison of the eluted fraction with the flow through from the ILY column. The eluted fraction (triangles) and the flow through (rectangles) from ILY column were dialyzed and concentrated to volume equal to amount of the human serum from (crosses) that was loaded on the ILY column. ILY concentration for hemolytic assay is 1.6×10^{-9} M. These data represent an experiment repeated three times.

Figure 2C is a western blot showing the isolation of ILY-binding proteins from human serum. The lanes were loaded as follows: 1: 0.8 μl of human serum, 2: 0.8 μl flow through from human serum loaded on the ILY-binding column, 3: 10 μg proteins from the eluted fraction of human serum loaded on ILY column, 4: 0.8 μl of mouse serum, 5: 0.8 μl flow through from mouse serum loaded on ILY-binding column, 6: 10 μg protein from the eluted fraction of mouse serum loaded on ILY column. Arrows indicate bands cut for protein sequencing.

Figure 2D is a table showing protein sequencing information.

Figure 3A is a western blot showing further isolation of ILY-binding human IgG with protein G column. Lanes were loaded as follows: 1: protein maker; 2: 10 μl of the fraction eluted from ILY column; 3: 20 μl of the eluted fraction after further purification with protein G column; 4: 20 μl of the flow through from further purification with protein G column. Samples used for lanes 2, 3, and 4 were equalized to the same volume by dialysis and concentration. Total original volume of the each sample of lane 2, lane 3, and lane 4 are equal.

Figure 3B is a graph showing percent ILY mediated hemolysis with the inhibitory effect of human ILY-binding IgG. The volumes of the eluted fraction from ILY column (crosses), the eluate from further purification with protein G column (rectangles), and the flow through from further purification with protein G column (triangles) were equalized by dialysis and concentration. ILY concentration for the hemolytic assay is 1.6×10^{-9} M. These data represent an experiment repeated three times.

Figure 3C is a graph showing percent ILY mediated hemolysis in the absence of an inhibitory effect of mouse ILY-binding IgG. The volumes of mouse and human serum loaded on the ILY-binding column were equal. The total volume of the eluted fraction from mouse serum (rectangles), flow through from mouse serum (crosses), and the eluted fraction from human serum (triangles) were equalized by dialysis and concentration. ILY concentration is 1.6×10^{-9} M.

Figure 3D is a graph showing ILY binding to the Fab region of human ILY-binding. 200 ng ILY was used to coat each well.

Figure 4A is a graph of percent lysis as a function of ILY concentration. These data show erythrocytes from ThCD59^{RBC} mice are hypersensitive to ILY-mediated cell lysis *ex vivo*. Results represent mean ± s.e.m. from $n = 4$ erythrocyte (RBC) samples per group. $^{*}P < 0.001$ versus wild type (WT).

Figure 4B is a graph showing percent lysis of the indicated cell type in response to the indicated amount of ILY. Human and ThCD59^{RBC} mouse RBCs are more sensitive to ILY-mediated lysis than WT erythrocytes. $^{*}P > 0.05$ versus WT for comparison of the percentage of lysis.

Figure 4C is a graph showing hematocrit as a function of time after ILY injection. Injection of a sublethal dose of ILY (45 ng per g body weight) induced a marked decrease in hematocrit values in ThCD59^{RBC} mice but not in WT mice (mean ± s.e.m. from $n = 4$ mice per group). $^{**}P < 0.001$, $^{*}P < 0.05$ versus WT.

Figure 4D is a graph showing percent reduction in hematocrit at the indicated dosage of ILY. ILY treatment induces dose-dependent intravascular hemolysis in ThCD59^{RBC} mice. $^{*}P < 0.001$ versus mice injected with ILY (30 ng per g body weight).

Figure 4E is a photograph and graph showing free hemoglobin in the indicated cells. ILY treatment (45 ng/g body weight) induces dose-dependent intravascular hemolysis in ThCD59^{RBC} mice. $^{*}P < 0.001$ versus WT injected with ILY. Hb, hemoglobin.

Figure 4F is a photograph and graph showing gross hemoglobinuria as a function of time after ILY injection. WT and ThCD59^{RBC} mice were injected with ILY (45 ng/g body weight). Urine was collected at various time points after injection (5 h after ILY injection and again after 1 d, 2 d, 3 d and 4 d). Gross hemoglobinuria is shown in the left panel; hemoglobin levels in the urine (1 to 4 dilution in PBS) are

shown in the right panel. Results shown in 4B, 4D, 4E and 4F are mean ± s.e.m. *P < 0.001 versus WT at day 1.

Figure 5A is a graph showing lysis as a function of ILY concentration in the indicated species. Hemolytic activity in human erythrocytes after addition of serial dilutions of ILY plus diluted sera (1:8 in PBS) from various species. Results represent mean from three independent experiments with different batches of purchased sera.

Figure 5B is a graph shwoign percent lysis as a function of dilution of serum. Hemolytic activity in human erythrocytes after addition of ILY (1.6×10^{-9} M) plus serially diluted human serum, eluate or flow-through from the ILY affinity column.

Figure 5C is a photograph of an SDS-Page gel showing protein fractions of t he indicated samples. Separation of serum, eluted fractions and flow-through from the ILY affinity column on 6% nonreducing SDS-PAGE. The ILY-binding fraction from human serum was further purified on a protein G affinity chromatography column (lanes 10 and 11). Lane 12 contains the human IgG standard.

Figure 5D is a graph showing percent lysis as a function of dilution of the indicated samples. Hemolytic activity in human erythrocytes after addition of ILY (1.6×10^{-9} M) plus serially diluted human serum, eluate or flow-through from the G protein column.

Figure 5E is a graph showing percent lysis as a function of dilution of the indicated samples. Hemolytic activity in human erythrocytes after addition of ILY (1.6×10^{-9} M) plus serially diluted mouse serum, eluate or flow-through from the ILY affinity column. Values shown in Figures 5B, 5D, and 5E are mean ± s.e.m. from three independent experiments per group. **P < 0.001, *P < 0.05 versus flow-through fraction.

Figure 6A is a graph showing percent survival mice treated with the indicated amount of ILY-binding IgG. Human ILY-binding IgG rescued ILY-induced acute death in ThCD59^{RBC} mice, but bovine ILY-binding IgG did not. Mice were injected intravenously with ILY-neutralizing human IgG and then injected with ILY (283.5 ng per g body weight, three times the lethal dose (LD_{100})) after 15 min.

Figure 6B is a graph showing hematocrit as a function of time after ILY injection in animals treated with the indicated IgG. ILY-neutralizing human IgG (1 μg per g body weight) rescued mice from intravascular hemolysis induced by ILY

injection (45 ng per g body weight) in ThCD59^{RBC} mice, but ILY-binding bovine IgG (1 μg per g body weight) did not. Results represent mean ± s.e.m. from $n = 5$ mice per group. *$P < 0.001$ versus corresponding human IgG group.

Figures 6C and 6D are graphs showing nitrate + nitrine or sP-Selectin concentration in the indicated samples. Mice were pretreated with either PBS or ILY-neutralizing human IgG (1 μg per g body weight), followed by ILY injection (45 ng per g body weight) after 15 min. WT/ILY: WT treated with PBS plus ILY; T/ILY: ThCD59^{RBC} mice treated with PBS plus ILY; T/ILY + Ab: ThCD59^{RBC} mice treated with ILY plus ILY-neutralizing human IgG. Results represent mean ± s.e.m. *$P <$ 0.001 versus WT/ILY or T/ILY + Ab.

Figure 7A is a photomicrograph showing endothelial cells. Mice are treated with ILY (3 ng per g body weight). Left panel shows two different circulating endothelial cells (solid arrows) and a particle of circulating endothelial cell (dashed arrow) stained with acridine. Right panel shows a phase-contrast image of a control. Scale bar, 20 μm.

Figure 7B is a graph showing circulating endothelial cells counts in the indicated sample. * $P < 0.01$ versus WT/ILY or T/ILY + Ab. Values shown are mean ± s.e.m.

Figure 7C is a photomicrograph of endothelial tissue showing endothelial damage, as detected by Evans blue staining. * $P < 0.001$ versus WT/ILY or T/ILY + Ab from $n = 4$ mice per group. Scale bar, 1 mm.

Figure 7D and 7E are photomicrographs of endothelial tissue. Ultrastructural features of endothelial damage in hepatic vessels. Note the typical chromatin condensation, irregular luminal membranes with numerous processes and blebs (grey arrows) and enlarged vacuoles in the dead endothelial cells (Figure 7D), and the absence of continuous basement membrane (inside the black circle) and widening of the intercellular spaces (star) (Figure 7E). EC, endothelium; V, vacuole. Black arrow points to basement membrane.

Figure 7F is a photomicrograph showing endothelial tissue. Endothelium does not show any visible alteration in its ultrastructure. EC, endothelium; Black arrow points to basement membrane. Scale bars in Figures 7D-7F=2 μm. Values shown are mean ± s.e.m.

Figure 7G is a graph showing absorption intensity of plasma in the indicated samples. Measurement of vWF in plasma by absorption intensity (OD_{450}). WT/ILY: WT treated with PBS plus ILY; T/ILY: $ThCD59^{RBC}$ mice treated with PBS plus ILY; T/ILY + Ab: $ThCD59^{RBC}$ mice treated with ILY-neutralizing human IgG plus ILY. $^*P < 0.01$ versus WT/ILY or T/ILY + Ab.

Figure 7H is a graph showing percent survival rate in the indicated mice. Survival rate of mice pretreated with either PBS or ILY-neutralizing human IgG (1 μg per g body weight) followed by ILY injection 15 min later (22.5 ng/g body weight, three times the LD_{100}). Values shown are mean ± s.e.m.

Figure 8A is a series of photomicrographs of endothelial tissue. ILY induces disseminated intravascular coagulation (DIC) features in $ThCD59^{END}$ mice but not in $ThCD59^{END}$ mice pretreated with ILY-neutralizing human IgG. DIC features included intra-alveolar edema (black arrow), perivascular inflammation (dark gray, right facing arrow) with neutrophil infiltration (white arrow), thrombosis (light gray, right facing arrows) and neutrophil margination (Left facing, gray arrow). Left column shows 20x magnification. Right column shows a 60x magnification of the area outlined by the black rectangle in the image at left. Scale bar, 40 μm.

Figure 8B is a graph showing concentration of TAT in the indicated samples. ILY resulted in increased TAT in $ThCD59^{END}$ mice but not in WT or $ThCD59^{END}$ mice pretreated with ILY-neutralizing human IgG.

Figure 8C is a graph showing platelet counts in the indicated samples. Decreased platelet counts in $ThCD59^{END}$ mice but not in WT or $ThCD59^{END}$ mice pretreated with ILY-neutralizing human IgG. WT/ILY: WT treated with PBS plus ILY; T/ILY: $ThCD59^{RBC}$ treated with PBS plus ILY; T/ILY + Ab: $ThCD59^{RBC}$ treated with ILY-neutralizing human IgG plus ILY. Values shown in 8B and 8C are mean ± s.e.m. $^*P < 0.01$ versus WT/ILY or T/ILY + Ab.

Figure 9A is a graph showing number of events as a function of fluorescence. FACS analysis was performed using the anti-hCD59 antibody, Bric 229, to examine hCD59 expression of $ThCD59^{RBC}$ erythrocytes. Red=WT erythrocytes; Green=human erythrocytes; Black=$ThCD59^{RBC}$ erythrocytes.

Figure 9B is graph showing percent lysis as a function of dilution. hCD59 expressed on erythrocytes from $ThCD59^{RBC}$ mice protected cells from MAC-mediated lysis in a dose-dependent manner. A mouse MAC-mediated hemolytic assay was

performed as previously described. Diamonds=WT erythrocytes; Large and small boxes=erythrocytes from two different lines of ThCD59^{RBC} mice expressing hCD59 at low and high levels, respectively. Results represent mean values ± s.e.m. from $n = 5$ mice per group. $^{*}P < 0.001$ *vs.* corresponding WT groups. $^{**}P < 0.05$ vs. corresponding ThCD59^{RBC} mice expressing hCD59 at the lower level (line).

Figure 9C is a photomicrograph of a northern blot showing RNA concentration. Northern blot analysis shows that hCD59 mRNA is present in the spleen and bone marrow only. Both 28S and 18S are shown as RNA loading controls.

Figure 9D is a photomicrograph of a northern blot showing RNA concentration. hCD59 mRNAs were detected in ThCD59^{END} mice.

Figure 9E is a series of fluorescent photomicrographs showing hCD59 protein. HCD59 protein was detected (gray arrows) in small size vessels and glomeruli of ThCD59^{END} but not of WT mice (immunofluorescence staining with anti-hCD59 antibody). Lower panels are phase-contrast controls for the corresponding upper panels. In the lower panels, the area inside the black circle is a renal glomerulus and the black arrows point to renal tubules. KG=Kidney glomeruli; KV=Kidney vein; and B=Brain. Scale bar, 50 μm.

Figure 10A and 10B are a graphs showing hematocrit in the indicated samples. The indicated i.p. injection. Mice (n = 3) died at 2-3 hours after i.p. injection of ILY. * P< 0.001 *vs*before injection. i.m. injection. Mice (n = 3) died at 7-9 hours after i.m. injection of ILY. * P< 0.01 *vs*before injection and ** P< 0.001 vsat 1 hour after injection.

Figure 11A-11C are fluorescent photomicrographs of the indicated tissue. Identification of ILY in tissue by double immunofluorescence: colocalizationof macrophage and recombinant ILY in(11A) brain, (11B) liver and (11C) lung. ILY: ILY injection. PBS: PBS injection. Macrophages were labeled by anti-CD68 and FITC-conjugated secondary antibodies. Recombinant ILY was labeled by anti-His tag and Rhodamine-conjugated secondary antibodies.

Detailed Description

The invention features compositions and methods for selectively killing CD59 expressing cells in a subject by administering ILY. These compositions and methods

are useful for treating conditions associated with cells expressing CD59, including proliferative diseases, obesity, and obesity related syndromes. These compositions can be used in combination with anti-hCD59 antibody, such that in desired locations, the lytic activity of ILY is suppressed by the antibody, and in other locations, where antibody is not applied, ILY lytic activity is permitted. This invention also features methods and compositions useful for preventing pregnancy.

Further, the invention features methods of killing specific target cells by administering ILY to a non-human animal containing a subpopulation of cells expressing hCD59.

I. Methods of Administration

The invention embodies local administration of ILY to the tissue of interest in a subject. The ILY can be formulated, for example, for topical, percutaneous, surgical, or transdermal administration. ILY may, for example, be formulated for sustained release.

II. Indications

Obesity

The methods and compositions of the invention are useful for treating obesity and obesity related syndromes. Examples of such syndromes include depression, type 2 diabetes, dyslipidemia, respiratory complications, sleep apnea, hypertension, gall bladder disease, heart disease (e.g., coronary artery disease), and ostheoarthritis.

The methods and compositions of this invention are also useful for reducing undesired adipose tissue.

In these methods, ILY is administered to the subject at the site of undesired CD59-expressing adipose cells.

Cancer

The compounds and methods of the invention are useful for the treatment of cancers and other disorders characterized by hyperproliferative cells. In these embodiments, ILY can be administered directly to an hCD59-expressing neoplasia, or systemically to a subject having a neoplasia characterized by the cell surface expression of hCD59.

Therapy may be performed alone or in conjunction with another therapy (e.g., surgery, radiation therapy, chemotherapy, immunotherapy, anti-angiogenesis therapy, or gene therapy). The duration of the therapy depends on the type of disease or disorder being treated, the age and condition of the patient, the stage and type of the patient's disease, and how the patient responds to the treatment. Therapy may be given in on-and-off cycles that include rest periods so that the patient's body has a chance to recovery from any as yet unforeseen side-effects. Desirably, the methods and compositions of the invention are more effective than other methods and compositions. By "more effective" is meant that a method, composition, or kit exhibits greater efficacy, is less toxic, safer, more convenient, better tolerated, or less expensive, or provides more treatment satisfaction than another method, composition, or kit with which it is being compared.

Cancers include, without limitation, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenroglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma). In a preferred embodiment, the proliferative disease is melanoma.

Skin-related conditions

The invention also features topical administration of ILY to treat skin-related disorders. Inflammatory skin-related disorders result in the damage of healthy skin tissue by an inflammatory process. Examples of inflammatory skin-related disorders include scleroderma, systemic lupus erythematosus, and inflammatory dermatoses. Inflammatory dermatoses include, for example, psoriasis, atopic dermatitis, non-specific dermatitis, lamellar ichthyosis, epidemlolytic hyperkeratosis, premalignant keratosis, acne, and seborrheic dermatitis, pityriasis roseas, acute febrile neutrophilic dermatosis, eczema (e.g., asteatotic eczema, dyshidrotic eczema, vesicular palmoplantar eczema), balanitis circumscripta plasmacellularis, balanoposthitis, Behcet disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, juvenile palmar-plantar dermatosis, keratosis pilaris, acne fulminans, acrodermatitis enteropathica, infantile acropustulosis, mastocytomas, diffuse cutaneous mastocytosis, erythema multiforme mino, erythema multiforme major, bullous dermatosis, alopecia, vitiligo, and transient acantholytic dermatosis. albinism, melasma, vitiligo, hair graying, freckles, hemochromatosis, hemosideriosis, and tinea versicolor.

The invention is also useful for treating intrinsic aging (i.e., chronological aging) as well as extrinsic aging (i.e., resulting from environmental conditions). Examples of such conditions include wrinkles (e.g., fine and coarse wrinkles), brown spots, dyspigmentation, laxity, yellow hue, telangiectasia, leathery appearance, lentigines, guttate hypomelanosis, solar keratoses, seborrhoeic keratoses, ephelides, actinic lentigo, and cutaneous malignancies.

ILY can be administered topically in combination with, for example, a neutralizing anti-ILY antibody. In this embodiment, the neutralizing anti-ILY antibody is administered portions of the skin where ILY-mediated cell death is undesired.

III. Topical Formulations

In the methods of the invention, ILY can be delivered to the skin in a topical formulation. Topical formulations include, without limitation, creams, lotions, gels,

sticks, ointments, sprays, foams, patches, aerosols, wound dressings, and drops. The formulations can be administered, for example, using a metered dose spray applicator, a micro-needle, iontophoresis, ultrasound penetration enhancement, electroporation, nano/micro-injection, sponge, or by applying and spreading the formulation by hand.

Any conventional pharmacologically and cosmetically acceptable vehicles may be used. For example, compounds may be administered in liposomal formulations that allow the biologically active compounds to enter the skin. Such liposomal formulations are described in, for example, U.S. Patent Nos. 5,169,637; 5,000,958; 5,049,388; 4,975,282; 5,194,266; 5,023,087; 5,688,525; 5,874,104; 5,409,704; 5,552,155; 5,356,633; 5,032,582; 4,994,213; and PCT Publication No. WO 96/40061. Examples of other appropriate vehicles are described in U.S. Patent No. 4,877,805 and EP Publication No. 0586106A1. Suitable vehicles of the invention may also include mineral oil, petrolatum, polydecene, stearic acid, isopropyl myristate, polyoxyl 40 stearate, stearyl alcohol, or vegetable oil.

The formulations can include various conventional colorants, fragrances, thickeners (e.g., xanthan gum), preservatives, humectants, emollients (e.g., hydrocarbon oils, waxes, or silicones), demulcents, emulsifying excipients, dispersants, penetration enhancers, plasticizing agents, preservatives, stabilizers, demulsifiers, wetting agents, emulsifiers, moisturizers, astringents, deodorants, and the like can be added to provide additional benefits and improve the feel and/or appearance of the topical preparation.

The topical formulations of the invention will typically have a pH of between 5.5 and 8.5 and include from about 0.000001% to 10% (w/v), desirably 0.001% to 0.1% (w/v), of the compounds of the invention.

Antioxidants

The formulations of the invention can also contain one or more antioxidants. Useful antioxidants include, without limitation, thiols (e.g., aurothioglucose, dihydrolipoic acid, propylthiouracil, thioredoxin, glutathione, cysteine, cystine, cystamine, thiodipropionic acid), sulphoximines (e.g., buthionine-sulphoximines, homo-cysteine-sulphoximine, buthionine-sulphones, and penta-, hexa- and heptathionine-sulphoximine), metal chelators (e.g, α-hydroxy-fatty acids, palmitic acid, phytic acid, lactoferrin, citric acid, lactic acid, and malic acid, humic acid, bile

acid, bile extracts, bilirubin, biliverdin, EDTA, EGTA, and DTPA), vitamins (e.g., vitamin E, vitamin C, ascorbyl palmitate, Mg ascorbyl phosphate, and ascorbyl acetate), phenols (e.g., butylhydroxytoluene, butylhydroxyanisole, ubiquinol, nordihydroguaiaretic acid, trihydroxybutyrophenone), benzoates (e.g., coniferyl benzoate), uric acid, mannose, propyl gallate, selenium (e.g., selenium-methionine), stilbenes (e.g., stilbene oxide and trans-stilbene oxide), and combinations thereof.

Antioxidants that may be incorporated into the formulations of the invention include natural antioxidants prepared from plant extracts, such as extracts from aloe vera; avocado; chamomile; echinacea; ginko biloba; ginseng; green tea; heather; jojoba; lavender; lemon grass; licorice; mallow; oats; peppermint; St. John's wort; willow; wintergreen; wheat wild yam extract; marine extracts; and mixtures thereof.

The total amount of antioxidant included in the formulations can be from 0.001% to 3% by weight, preferably 0.01% to 1% by weight, in particular 0.05% to 0.5% by weight, based on the total weight of the formulation.

Emulsifying Excipients

Formulations of the invention can further include one or more emulsifying excipients. Emulsifying excipients that may be used in the formulations of the invention include, without limitation, compounds belonging to the following classes: polyethoxylated fatty acids, PEG-fatty acid diesters, PEG-fatty acid mono-ester and di-ester mixtures, polyethylene glycol glycerol fatty acid esters, alcohol-oil transesterification products, polyglycerized fatty acids, propylene glycol fatty acid esters, mixtures of propylene glycol esters and glycerol esters, mono- and diglycerides, sterol and sterol derivatives, polyethylene glycol sorbitan fatty acid esters, polyethylene glycol alkyl ethers, sugar esters, polyethylene glycol alkyl phenols, polyoxyethylene-polyoxypropylene block copolymers, sorbitan fatty acid esters, lower alcohol fatty acid esters, ionic surfactants, tocopherol esters, and sterol esters. Commercially available examples for each class of excipient are provided below.

Polyethoxylated fatty acids may be used as excipients for the formulations of the invention. Examples of commercially available polyethoxylated fatty acid monoester surfactants include: PEG 4-100 monolaurate (Crodet L series, Croda), PEG 4-100 monooleate (Crodet O series, Croda), PEG 4-100 monostearate (Crodet S series, Croda, and Myrj Series, Atlas/ICI), PEG 400 distearate (Cithrol 4DS series, Croda),

PEG 100, 200, or 300 monolaurate (Cithrol ML series, Croda), PEG 100, 200, or 300 monooleate (Cithrol MO series, Croda), PEG 400 dioleate (Cithrol 4DO series, Croda), PEG 400-1000 monostearate (Cithrol MS series, Croda), PEG-1 stearate (Nikkol MYS-1EX, Nikko, and Coster K1, Condea), PEG-2 stearate (Nikkol MYS-2, Nikko), PEG-2 oleate (Nikkol MYO-2, Nikko), PEG-4 laurate (Mapeg® 200 ML, PPG), PEG-4 oleate (Mapeg® 200 MO, PPG), PEG-4 stearate (Kessco® PEG 200 MS, Stepan), PEG-5 stearate (Nikkol TMGS-5, Nikko), PEG-5 oleate (Nikkol TMGO-5, Nikko), PEG-6 oleate (Algon OL 60, Auschem SpA), PEG-7 oleate (Algon OL 70, Auschem SpA), PEG-6 laurate (Kessco® PEG300 ML, Stepan), PEG-7 laurate (Lauridac 7, Condea), PEG-6 stearate (Kessco® PEG300 MS, Stepan), PEG-8 laurate (Mapeg® 400 ML, PPG), PEG-8 oleate (Mapeg® 400 MO, PPG), PEG-8 stearate (Mapeg® 400 MS, PPG), PEG-9 oleate (Emulgante A9, Condea), PEG-9 stearate (Cremophor S9, BASF), PEG-10 laurate (Nikkol MYL-10, Nikko), PEG-10 oleate (Nikkol MYO-10, Nikko), PEG-12 stearate (Nikkol MYS-10, Nikko), PEG-12 laurate (Kessco® PEG 600 ML, Stepan), PEG-12 oleate (Kessco® PEG 600 MO, Stepan), PEG-12 ricinoleate (CAS # 9004-97-1), PEG-12 stearate (Mapeg® 600 MS, PPG), PEG-15 stearate (Nikkol TMGS-15, Nikko), PEG-15 oleate (Nikkol TMGO-15, Nikko), PEG-20 laurate (Kessco® PEG 1000 ML, Stepan), PEG-20 oleate (Kessco® PEG 1000 MO, Stepan), PEG-20 stearate (Mapeg® 1000 MS, PPG), PEG-25 stearate (Nikkol MYS-25, Nikko), PEG-32 laurate (Kessco® PEG 1540 ML, Stepan), PEG-32 oleate (Kessco® PEG 1540 MO, Stepan), PEG-32 stearate (Kessco® PEG 1540 MS, Stepan), PEG-30 stearate (Myrj 51), PEG-40 laurate (Crodet L40, Croda), PEG-40 oleate (Crodet O40, Croda), PEG-40 stearate (Emerest® 2715, Henkel), PEG-45 stearate (Nikkol MYS-45, Nikko), PEG-50 stearate (Myrj 53), PEG-55 stearate (Nikkol MYS-55, Nikko), PEG-100 oleate (Crodet O-100, Croda), PEG-100 stearate (Ariacel 165, ICI), PEG-200 oleate (Albunol 200 MO, Taiwan Surf.), PEG-400 oleate (LACTOMUL, Henkel), and PEG-600 oleate (Albunol 600 MO, Taiwan Surf.). Formulations of the invention may include one or more of the polyethoxylated fatty acids above.

Polyethylene glycol fatty acid diesters may be used as excipients for the formulations of the invention. Examples of commercially available polyethylene glycol fatty acid diesters include: PEG-4 dilaurate (Mapeg® 200 DL, PPG), PEG-4 dioleate (Mapeg® 200 DO, PPG), PEG-4 distearate (Kessco® 200 DS, Stepan), PEG-

6 dilaurate (Kessco® PEG 300 DL, Stepan), PEG-6 dioleate (Kessco® PEG 300 DO, Stepan), PEG-6 distearate (Kessco® PEG 300 DS, Stepan), PEG-8 dilaurate (Mapeg® 400 DL, PPG), PEG-8 dioleate (Mapeg® 400 DO, PPG), PEG-8 distearate (Mapeg® 400 DS, PPG), PEG-10 dipalmitate (Polyaldo 2PKFG), PEG-12 dilaurate (Kessco® PEG 600 DL, Stepan), PEG-12 distearate (Kessco® PEG 600 DS, Stepan), PEG-12 dioleate (Mapeg® 600 DO, PPG), PEG-20 dilaurate (Kessco® PEG 1000 DL, Stepan), PEG-20 dioleate (Kessco® PEG 1000 DO, Stepan), PEG-20 distearate (Kessco® PEG 1000 DS, Stepan), PEG-32 dilaurate (Kessco® PEG 1540 DL, Stepan), PEG-32 dioleate (Kessco® PEG 1540 DO, Stepan), PEG-32 distearate (Kessco® PEG 1540 DS, Stepan), PEG-400 dioleate (Cithrol 4DO series, Croda), and PEG-400 distearate Cithrol 4DS series, Croda). Formulations of the invention may include one or more of the polyethylene glycol fatty acid diesters above.

PEG-fatty acid mono- and di-ester mixtures may be used as excipients for the formulations of the invention. Examples of commercially available PEG-fatty acid mono- and di-ester mixtures include: PEG 4-150 mono, dilaurate (Kessco® PEG 200-6000 mono, Dilaurate, Stepan), PEG 4-150 mono, dioleate (Kessco® PEG 200-6000 mono, Dioleate, Stepan), and PEG 4-150 mono, distearate (Kessco® 200-6000 mono, Distearate, Stepan). Formulations of the invention may include one or more of the PEG-fatty acid mono- and di-ester mixtures above.

Polyethylene glycol glycerol fatty acid esters may be used as excipients for the formulations of the invention. Examples of commercially available polyethylene glycol glycerol fatty acid esters include: PEG-20 glyceryl laurate (Tagat® L, Goldschmidt), PEG-30 glyceryl laurate (Tagat® L2, Goldschmidt), PEG-15 glyceryl laurate (Glycerox L series, Croda), PEG-40 glyceryl laurate (Glycerox L series, Croda), PEG-20 glyceryl stearate (Capmul® EMG, ABITEC), and Aldo® MS-20 KFG, Lonza), PEG-20 glyceryl oleate (Tagat® O, Goldschmidt), and PEG-30 glyceryl oleate (Tagat® O2, Goldschmidt). Formulations of the invention may include one or more of the polyethylene glycol glycerol fatty acid esters above.

Alcohol-oil transesterification products may be used as excipients for the formulations of the invention. Examples of commercially available alcohol-oil transesterification products include: PEG-3 castor oil (Nikkol CO-3, Nikko), PEG-5, 9, and 16 castor oil (ACCONON CA series, ABITEC), PEG-20 castor oil, (Emalex C-20, Nihon Emulsion), PEG-23 castor oil (Emulgante EL23), PEG-30 castor oil

(Incrocas 30, Croda), PEG-35 castor oil (Incrocas-35, Croda), PEG-38 castor oil (Emulgante EL 65, Condea), PEG-40 castor oil (Emalex C-40, Nihon Emulsion), PEG-50 castor oil (Emalex C-50, Nihon Emulsion), PEG-56 castor oil (Eumulgin® PRT 56, Pulcra SA), PEG-60 castor oil (Nikkol CO-60TX, Nikko), PEG-100 castor oil, PEG-200 castor oil (Eumulgin® PRT 200, Pulcra SA), PEG-5 hydrogenated castor oil (Nikkol HCO-5, Nikko), PEG-7 hydrogenated castor oil (Cremophor WO7, BASF), PEG-10 hydrogenated castor oil (Nikkol HCO-10, Nikko), PEG-20 hydrogenated castor oil (Nikkol HCO-20, Nikko), PEG-25 hydrogenated castor oil (Simulsol® 1292, Seppic), PEG-30 hydrogenated castor oil (Nikkol HCO-30, Nikko), PEG-40 hydrogenated castor oil (Cremophor RH 40, BASF), PEG-45 hydrogenated castor oil (Cerex ELS 450, Auschem Spa), PEG-50 hydrogenated castor oil (Emalex HC-50, Nihon Emulsion), PEG-60 hydrogenated castor oil (Nikkol HCO-60, Nikko), PEG-80 hydrogenated castor oil (Nikkol HCO-80, Nikko), PEG-100 hydrogenated castor oil (Nikkol HCO-100, Nikko), PEG-6 corn oil (Labrafil® M 2125 CS, Gattefosse), PEG-6 almond oil (Labrafil® M 1966 CS, Gattefosse), PEG-6 apricot kernel oil (Labrafil® M 1944 CS, Gattefosse), PEG-6 olive oil (Labrafil® M 1980 CS, Gattefosse), PEG-6 peanut oil (Labrafil® M 1969 CS, Gattefosse), PEG-6 hydrogenated palm kernel oil (Labrafil® M 2130 BS, Gattefosse), PEG-6 palm kernel oil (Labrafil® M 2130 CS, Gattefosse), PEG-6 triolein (Labrafil® M 2735 CS, Gattefosse), PEG-8 corn oil (Labrafil® WL 2609 BS, Gattefosse), PEG-20 corn glycerides (Crovol M40, Croda), PEG-20 almond glycerides (Crovol A40, Croda), PEG-25 trioleate (TAGAT® TO, Goldschmidt), PEG-40 palm kernel oil (Crovol PK-70), PEG-60 corn glycerides (Crovol M70, Croda), PEG-60 almond glycerides (Crovol A70, Croda), PEG-4 caprylic/capric triglyceride (Labrafac® Hydro, Gattefosse), PEG-8 caprylic/capric glycerides (Labrasol, Gattefosse), PEG-6 caprylic/capric glycerides (SOFTIGEN®767, Huls), lauroyl macrogol-32 glyceride (GELUCIRE 44/14, Gattefosse), stearoyl macrogol glyceride (GELUCIRE 50/13, Gattefosse), mono, di, tri, tetra esters of vegetable oils and sorbitol (SorbitoGlyceride, Gattefosse), pentaerythrityl tetraisostearate (Crodamol PTIS, Croda), pentaerythrityl distearate (Albunol DS, Taiwan Surf.), pentaerythrityl tetraoleate (Liponate PO-4, Lipo Chem.), pentaerythrityl tetrastearate (Liponate PS-4, Lipo Chem.), pentaerythrityl tetracaprylate tetracaprate (Liponate PE-810, Lipo Chem.), and pentaerythrityl tetraoctanoate (Nikkol Pentarate 408, Nikko). Also included as oils in

this category of surfactants are oil-soluble vitamins, such as vitamins A, D, E, K, etc. Thus, derivatives of these vitamins, such as tocopheryl PEG-1000 succinate (TPGS, available from Eastman), are also suitable surfactants. Formulations of the invention may include one or more of the alcohol-oil transesterification products above.

Polyglycerized fatty acids may be used as excipients for the formulations of the invention. Examples of commercially available polyglycerized fatty acids include: polyglyceryl-2 stearate (Nikkol DGMS, Nikko), polyglyceryl-2 oleate (Nikkol DGMO, Nikko), polyglyceryl-2 isostearate (Nikkol DGMIS, Nikko), polyglyceryl-3 oleate (Caprol® 3GO, ABITEC), polyglyceryl-4 oleate (Nikkol Tetraglyn 1-O, Nikko), polyglyceryl-4 stearate (Nikkol Tetraglyn 1-S, Nikko), polyglyceryl-6 oleate (Drewpol 6-1-O, Stepan), polyglyceryl-10 laurate (Nikkol Decaglyn 1-L, Nikko), polyglyceryl-10 oleate (Nikkol Decaglyn 1-O, Nikko), polyglyceryl-10 stearate (Nikkol Decaglyn 1-S, Nikko), polyglyceryl-6 ricinoleate (Nikkol Hexaglyn PR-15, Nikko), polyglyceryl-10 linoleate (Nikkol Decaglyn 1-LN, Nikko), polyglyceryl-6 pentaoleate (Nikkol Hexaglyn 5-O, Nikko), polyglyceryl-3 dioleate (Cremophor GO32, BASF), polyglyceryl-3 distearate (Cremophor GS32, BASF), polyglyceryl-4 pentaoleate (Nikkol Tetraglyn 5-O, Nikko), polyglyceryl-6 dioleate (Caprol® 6G20, ABITEC), polyglyceryl-2 dioleate (Nikkol DGDO, Nikko), polyglyceryl-10 trioleate (Nikkol Decaglyn 3-O, Nikko), polyglyceryl-10 pentaoleate (Nikkol Decaglyn 5-O, Nikko), polyglyceryl-10 septaoleate (Nikkol Decaglyn 7-O, Nikko), polyglyceryl-10 tetraoleate (Caprol® 10G4O, ABITEC), polyglyceryl-10 decaisostearate (Nikkol Decaglyn 10-IS, Nikko), polyglyceryl-101 decaoleate (Drewpol 10-10-O, Stepan), polyglyceryl-10 mono, dioleate (Caprol® PGE 860, ABITEC), and polyglyceryl polyricinoleate (Polymuls, Henkel). Formulations of the invention may include one or more of the polyglycerized fatty acids above.

Propylene glycol fatty acid esters may be used as excipients for the formulations of the invention. Examples of commercially available propylene glycol fatty acid esters include: propylene glycol monocaprylate (Capryol 90, Gattefosse), propylene glycol monolaurate (Lauroglycol 90, Gattefosse), propylene glycol oleate (Lutrol OP2000, BASF), propylene glycol myristate (Mirpyl), propylene glycol monostearate (LIPO PGMS, Lipo Chem.), propylene glycol hydroxystearate, propylene glycol ricinoleate (PROPYMULS, Henkel), propylene glycol isostearate, propylene glycol monooleate (Myverol P-O6, Eastman), propylene glycol dicaprylate

dicaprate (Captex® 200, ABITEC), propylene glycol dioctanoate (Captex® 800, ABITEC), propylene glycol caprylate caprate (LABRAFAC PG, Gattefosse), propylene glycol dilaurate, propylene glycol distearate (Kessco® PGDS, Stepan), propylene glycol dicaprylate (Nikkol Sefsol 228, Nikko), and propylene glycol dicaprate (Nikkol PDD, Nikko). Formulations the invention may include one or more of the propylene glycol fatty acid esters above.

Mixtures of propylene glycol esters and glycerol esters may be used as excipients for the formulations of the invention. One preferred mixture is composed of the oleic acid esters of propylene glycol and glycerol (Arlacel 186). Examples of these surfactants include: oleic (ATMOS 300, ARLACEL 186, ICI), stearic (ATMOS 150). Formulations of the invention may include one or more of the mixtures of propylene glycol esters and glycerol esters above.

Mono- and diglycerides may be used as excipients for the formulations of the invention. Examples of commercially available mono- and diglycerides include: monopalmitolein (C16:1) (Larodan), monoelaidin (C18:1) (Larodan), monocaproin (C6) (Larodan), monocaprylin (Larodan), monocaprin (Larodan), monolaurin (Larodan), glyceryl monomyristate (C14) (Nikkol MGM, Nikko), glyceryl monooleate (C18:1) (PECEOL, Gattefosse), glyceryl monooleate (Myverol, Eastman), glycerol monooleate/linoleate (OLICINE, Gattefosse), glycerol monolinoleate (Maisine, Gattefosse), glyceryl ricinoleate (Softigen® 701, Huls), glyceryl monolaurate (ALDO® MLD, Lonza), glycerol monopalmitate (Emalex GMS-P, Nihon), glycerol monostearate (Capmul® GMS, ABITEC), glyceryl mono- and dioleate (Capmul® GMO-K, ABITEC), glyceryl palmitic/stearic (CUTINA MD-A, ESTAGEL-G18), glyceryl acetate (Lamegin® EE, Grunau GmbH), glyceryl laurate (Imwitor® 312, Huls), glyceryl citrate/lactate/oleate/linoleate (Imwitor® 375, Huls), glyceryl caprylate (Imwitor® 308, Huls), glyceryl caprylate/caprate (Capmul® MCM, ABITEC), caprylic acid mono- and diglycerides (Imwitor® 988, Huls), caprylic/capric glycerides (Imwitor® 742, Huls), Mono-and diacetylated monoglycerides (Myvacet® 9-45, Eastman), glyceryl monostearate (Aldo® MS, Arlacel 129, ICI), lactic acid esters of mono and diglycerides (LAMEGIN GLP, Henkel), dicaproin (C6) (Larodan), dicaprin (C10) (Larodan), dioctanoin (C8) (Larodan), dimyristin (C14) (Larodan), dipalmitin (C16) (Larodan), distearin (Larodan), glyceryl dilaurate (C12) (Capmul® GDL, ABITEC), glyceryl dioleate

(Capmul® GDO, ABITEC), glycerol esters of fatty acids (GELUCIRE 39/01, Gattefosse), dipalmitolein (C16:1) (Larodan), 1,2 and 1,3-diolein (C18:1) (Larodan), dielaidin (C18:1) (Larodan), and dilinolein (C18:2) (Larodan). Formulations of the invention may include one or more of the mono- and diglycerides above.

Sterol and sterol derivatives may be used as excipients for the formulations of the invention. Examples of commercially available sterol and sterol derivatives include: cholesterol, sitosterol, lanosterol, PEG-24 cholesterol ether (Solulan C-24, Amerchol), PEG-30 cholestanol (Phytosterol GENEROL series, Henkel), PEG-25 phytosterol (Nikkol BPSH-25, Nikko), PEG-5 soyasterol (Nikkol BPS-5, Nikko), PEG-10 soyasterol (Nikkol BPS-10, Nikko), PEG-20 soyasterol (Nikkol BPS-20, Nikko), and PEG-30 soyasterol (Nikkol BPS-30, Nikko). Formulations of the invention may include one or more of the sterol and sterol derivatives above.

Polyethylene glycol sorbitan fatty acid esters may be used as excipients for the formulations of the inveniton. Examples of commercially available polyethylene glycol sorbitan fatty acid esters include: PEG-10 sorbitan laurate (Liposorb L-10, Lipo Chem.), PEG-20 sorbitan monolaurate (Tween® 20, Atlas/ICI), PEG-4 sorbitan monolaurate (Tween® 21, Atlas/ICI), PEG-80 sorbitan monolaurate (Hodag PSML-80, Calgene), PEG-6 sorbitan monolaurate (Nikkol GL-1, Nikko), PEG-20 sorbitan monopalmitate (Tween® 40, Atlas/ICI), PEG-20 sorbitan monostearate (Tween® 60, Atlas/ICI), PEG-4 sorbitan monostearate (Tween® 61, Atlas/ICI), PEG-8 sorbitan monostearate (DACOL MSS, Condea), PEG-6 sorbitan monostearate (Nikkol TS106, Nikko), PEG-20 sorbitan tristearate (Tween® 65, Atlas/ICI), PEG-6 sorbitan tetrastearate (Nikkol GS-6, Nikko), PEG-60 sorbitan tetrastearate (Nikkol GS-460, Nikko), PEG-5 sorbitan monooleate (Tween® 81, Atlas/ICI), PEG-6 sorbitan monooleate (Nikkol TO-106, Nikko), PEG-20 sorbitan monooleate (Tween® 80, Atlas/ICI), PEG-40 sorbitan oleate (Emalex ET 8040, Nihon Emulsion), PEG-20 sorbitan trioleate (Tween® 85, Atlas/ICI), PEG-6 sorbitan tetraoleate (Nikkol GO-4, Nikko), PEG-30 sorbitan tetraoleate (Nikkol GO-430, Nikko), PEG-40 sorbitan tetraoleate (Nikkol GO-440, Nikko), PEG-20 sorbitan monoisostearate (Tween® 120, Atlas/ICI), PEG sorbitol hexaoleate (Atlas G-1086, ICI), polysorbate 80 (Tween® 80, Pharma), polysorbate 85 (Tween® 85, Pharma), polysorbate 20 (Tween® 20, Pharma), polysorbate 40 (Tween® 40, Pharma), polysorbate 60 (Tween® 60, Pharma), and PEG-6 sorbitol hexastearate (Nikkol GS-6, Nikko). Formulations of the

invention may include one or more of the polyethylene glycol sorbitan fatty acid esters above.

Polyethylene glycol alkyl ethers may be used as excipients for the formulations of the invention. Examples of commercially available polyethylene glycol alkyl ethers include: PEG-2 oleyl ether, oleth-2 (Brij 92/93, Atlas/ICI), PEG-3 oleyl ether, oleth-3 (Volpo 3, Croda), PEG-5 oleyl ether, oleth-5 (Volpo 5, Croda), PEG-10 oleyl ether, oleth-10 (Volpo 10, Croda), PEG-20 oleyl ether, oleth-20 (Volpo 20, Croda), PEG-4 lauryl ether, laureth-4 (Brij 30, Atlas/ICI), PEG-9 lauryl ether, PEG-23 lauryl ether, laureth-23 (Brij 35, Atlas/ICI), PEG-2 cetyl ether (Brij 52, ICI), PEG-10 cetyl ether (Brij 56, ICI), PEG-20 cetyl ether (BriJ 58, ICI), PEG-2 stearyl ether (Brij 72, ICI), PEG-10 stearyl ether (Brij 76, ICI), PEG-20 stearyl ether (Brij 78, ICI), and PEG-100 stearyl ether (Brij 700, ICI). Formulations of the invention may include one or more of the polyethylene glycol alkyl ethers above.

Sugar esters may be used as excipients for the formulations of the invention. Examples of commercially available sugar esters include: sucrose distearate (SUCRO ESTER 7, Gattefosse), sucrose distearate/monostearate (SUCRO ESTER 11, Gattefosse), sucrose dipalmitate, sucrose monostearate (Crodesta F-160, Croda), sucrose monopalmitate (SUCRO ESTER 15, Gattefosse), and sucrose monolaurate (Saccharose monolaurate 1695, Mitsubisbi-Kasei). Formulations of the invention may include one or more of the sugar esters above.

Polyethylene glycol alkyl phenols may be used as excipients for the formulations of the invention. Examples of commercially available polyethylene glycol alkyl phenols include: PEG-10-100 nonylphenol series (Triton X series, Rohm & Haas) and PEG-15-100 octylphenol ether series (Triton N-series, Rohm & Haas). Formulations of the invention may include one or more of the polyethylene glycol alkyl phenols above.

Polyoxyethylene-polyoxypropylene block copolymers may be used as excipients for the formulations of the invention. These surfactants are available under various trade names, including one or more of Synperonic PE series (ICI), Pluronic® series (BASF), Lutrol (BASF), Supronic, Monolan, Pluracare, and Plurodac. The generic term for these polymers is "poloxamer" (CAS 9003-11-6). These polymers have the formula I:

$$HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH \quad (I)$$

where "a" and "b" denote the number of polyoxyethylene and polyoxypropylene units, respectively. Formulations of the invention may include one or more of the polyoxyethylene-polyoxypropylene block copolymers above.

Polyoxyethylenes, such as PEG 300, PEG 400, and PEG 600, may be used as excipients for the formulations of the invention.

Sorbitan fatty acid esters may be used as excipients for the formulations of the invention. Examples of commercially sorbitan fatty acid esters include: sorbitan monolaurate (Span-20, Atlas/ICI), sorbitan monopalmitate (Span-40, Atlas/ICI), sorbitan monooleate (Span-80, Atlas/ICI), sorbitan monostearate (Span-60, Atlas/ICI), sorbitan trioleate (Span-85, Atlas/ICI), sorbitan sesquioleate (Arlacel-C, ICI), sorbitan tristearate (Span-65, Atlas/ICI), sorbitan monoisostearate (Crill 6, Croda), and sorbitan sesquistearate (Nikkol SS-15, Nikko). Formulations of the invention may include one or more of the sorbitan fatty acid esters above.

Esters of lower alcohols (C2 to C4) and fatty acids (C8 to C18) are suitable surfactants for use in the invention. Examples of these surfactants include: ethyl oleate (Crodamol EO, Croda), isopropyl myristate (Crodamol IPM, Croda), isopropyl palmitate (Crodamol IPP, Croda), ethyl linoleate (Nikkol VF-E, Nikko), and isopropyl linoleate (Nikkol VF-IP, Nikko). Formulations of the invention may include one or more of the lower alcohol fatty acid esters above.

Ionic surfactants may be used as excipients for the formulations of the invention. Examples of useful ionic surfactants include: sodium caproate, sodium caprylate, sodium caprate, sodium laurate, sodium myristate, sodium myristolate, sodium palmitate, sodium palmitoleate, sodium oleate, sodium ricinoleate, sodium linoleate, sodium linolenate, sodium stearate, sodium lauryl sulfate (dodecyl), sodium tetradecyl sulfate, sodium lauryl sarcosinate, sodium dioctyl sulfosuccinate, sodium cholate, sodium taurocholate, sodium glycocholate, sodium deoxycholate, sodium taurodeoxycholate, sodium glycodeoxycholate, sodium ursodeoxycholate, sodium chenodeoxycholate, sodium taurochenodeoxycholate, sodium glyco cheno deoxycholate, sodium cholylsarcosinate, sodium N-methyl taurocholate, egg yolk phosphatides, hydrogenated soy lecithin, dimyristoyl lecithin, lecithin, hydroxylated lecithin, lysophosphatidylcholine, cardiolipin, sphingomyelin, phosphatidylcholine, phosphatidyl ethanolamine, phosphatidic acid, phosphatidyl glycerol, phosphatidyl serine, diethanolamine, phospholipids, polyoxyethylene-10 oleyl ether phosphate,

esterification products of fatty alcohols or fatty alcohol ethoxylates, with phosphoric acid or anhydride, ether carboxylates (by oxidation of terminal OH group of, fatty alcohol ethoxylates), succinylated monoglycerides, sodium stearyl fumarate, stearoyl propylene glycol hydrogen succinate, mono/diacetylated tartaric acid esters of mono- and diglycerides, citric acid esters of mono-, diglycerides, glyceryl-lacto esters of fatty acids, acyl lactylates, lactylic esters of fatty acids, sodium stearoyl-2-lactylate, sodium stearoyl lactylate, alginate salts, propylene glycol alginate, ethoxylated alkyl sulfates, alkyl benzene sulfones, α-olefin sulfonates, acyl isethionates, acyl taurates, alkyl glyceryl ether sulfonates, sodium octyl sulfosuccinate, sodium undecylenamideo-MEA-sulfosuccinate, hexadecyl triammonium bromide, decyl trimethyl ammonium bromide, cetyl trimethyl ammonium bromide, dodecyl ammonium chloride, alkyl benzyldimethylammonium salts, diisobutyl phenoxyethoxydimethyl benzylammonium salts, alkylpyridinium salts, betaines (trialkylglycine), lauryl betaine (N-lauryl,N,N-dimethylglycine), and ethoxylated amines (polyoxyethylene-15 coconut amine). For simplicity, typical counterions are provided above. It will be appreciated by one skilled in the art, however, that any bioacceptable counterion may be used. For example, although the fatty acids are shown as sodium salts, other cation counterions can also be used, such as, for example, alkali metal cations or ammonium. Formulations of the invention may include one or more of the ionic surfactants above.

Tocopherol esters and sterol esters, as described in U.S. Patent Nos. 6,632,443 and 6,191,172, each of which is incorporated herein by reference, may be used as excipients for the formulations of the invention. These tocopherol and sterol esters are described by formula II:

$$\{X\text{-}OOC\text{-}[(CH_2)_n\text{-}COO]_m\}_p\text{-}Y \quad (II)$$

wherein X is selected from α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, cholesterol, 7-dehydrocholesterol, campesterol, sitosterol, ergosterol, and stigmasterol; p is 1 or 2; m is 0 or 1; n is an integer from 0 to 18; and Y is a hydrophilic moiety selected from polyalcohols, polyethers, and derivatives thereof.

The emulsifying excipients present in the formulations of the invention are present in amounts such that the carrier forms uniform dispersion of compounds of the invention. The relative amounts of surfactants required are readily determined by observing the properties of the resultant dispersion, as determined using standard

techniques for measuring solubilities. The optical clarity of the aqueous dispersion can be measured using standard quantitative techniques for turbidity assessment. For example, a formulation of the invention can include from 0.001% to 10% by weight, preferably 0.01% to 5% by weight, emulsifying excipient.

Gelling Agents

Formulations of the invention can also contain one or more gelling agents. Useful gelling agents include, without limitation, hydroxyethylcellulose (commercially available as NATROSOL® hydroxyethylcellulose produced by Aqualon), hydroxypropylcellulose (commercially available as KLUCEL® hydroxypropylcellulose produced by Aqualon), cross-linked acrylic acid polymers (such as the commercially available product CARBOPOL® cross linked acrylic acid polymer, produced by Goodrich), MVE/MA decadiene crosspolymer (such as the commercially available product STABILEZE® MVE/MA decadiene crosspolymer, produced by ISP), PVM/MA copolymer (such as the commercially available product GANTREZ® PVM/MA copolymer, produced by ISP), ammonium acrylates/acrylonitrogens (commercially available as HYPAN® ammonium acrylates/acrylonitrogens), carboxymethylcellulose, polyvinylpyrrolidone, carbomer (carboxypolymethylene, CAS 541823-57-9; of which different grades with various molecular weights are commercially available), cetostearyl alcohol, colloidal silicon dioxide, gelatin, guar gum, sodium or calcium carboxymethyl cellulose, hydroxyethyl or hydroxypropyl cellulose, hydroxypropylmethylcellulose, methyl or ethyl cellulose, maltodextrin, polyvinyl alcohol, propylene carbonate, povidone, propylene glycol alginate, alginic acid sodium alginate, sodium starch glycolate, starch, and sucrose. Typically, the gelling agent, when used, is present in an amount between about 0.5% to about 10% by weight of the composition. More particularly, for CARBOPOL® cross linked acrylic acid polymer the preferred compositional weight percent range is between about 2% to about 6%, while for NATROSOL® hydroxyethylcellulose or KLUCEL® hydroxypropylcellulose the preferred range is between about 0.5% to about 4%. Desirably, the compositional weight percent range for STABILEZE® PVM/MA decadiene crosspolymer and HYPAN® ammonium acrylates/acrylonitrogens is between about 1% to about 4%. The preferred

compositional weight percent range for polyvinylpyrrolidone is between about 0.5% and about 10%.

Hydrocolloids

Formulations of the invention can contain one or more hydrocolloids. Useful hydrocolloids include, without limitation, Carbopol, including Carbopol 940, carrageenan, agar, xanthan gum, locust bean gum polyglucomannan, and gelatin.

Cross-Linking Agents

Formulations of the invention can contain one or more cross-linking agents to form a chemical bond between the molecules of the polymer to gel the dispersion, forming a solid body. Examples of cross-linking agents for locust bean gum, guar or chemically modified guar are galactose, organic titanate or boric acid. When the hydrocolloid is a polyglucomannan (e.g., Konjak®), borax can be used as a cross-linking agent. When xanthan gum is used, a suitable cross-linker for xanthan gum is mannose. If locust bean gum is used as the principle hydrocolloid, lactose or other suitable oligosaccharide can be used.

Plasticizers

Formulations of the invention can contain one or more plasticizers. Useful plasticizers include, without limitation, alkyl glycols, polyalkylene glycols (e.g., polyethylene glycol and/or polypropylene glycol), benzyl benzoate, chlorobutanol, mineral oil, (CTFA mixture of mineral oils, e.g., Amerchol L-101, Protalan M-16, Protalan M-26), petrolatum (CTFA, mixture of petrolatum, e.g., Amerchol CAB, Forlan 200), lanolin alcohols, sorbitol, triacetin, dibutyl sebacate, diethyl phthalate, glycerine, petrolactam and triethyl citrate.

IV. Contraception

The present invention also includes compositions and methods for preventing pregnancy. The invention includes compositions containing ILY, i.e., the active ingredient, by itself or together with one or more pharmaceutically acceptable carriers, excipients or diluents, and, optionally, other prophylactic ingredients formulated for use as a constraceptive. The pharmaceutical formulations may, where appropriate, be

presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All such methods include the step of bringing into association the active ingredient (ILY) with liquid carriers or finely divided solid carriers.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, jelly, foams, sprays, aqueous, oily suspensions, solutions, emulsions (liquid formulations), or films. These formulations may contain, in addition to ILY, such carriers as are known in the art to be appropriate.

Liquid preparations according to the present invention would include ILY in a pharmaceutically acceptable liquid carrier or diluent, such as purified water or a physiological saline solution. The liquid preparations may also contain conventional additives, such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils) or preservatives.

Ointments, pastes, jellies, liquids, foams, gels and creams may, for example, be formulated with an aqueous or oil base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous base or an oil base and will in general also contain one or more emulsifying agents, coloring agents, stabilizing agents, suspending agents, thickening agents or surfactants, such as a nonionic surfactant, for example, a polyoxyethylene higher alcohol ether or polyethylene glycol.

A gel preparation having a high viscosity can be prepared by adding a conventional thickening agent into the above described liquid preparation. Non-limiting examples of thickening agents include cellulose lower alcohol ether, polyvinyl alcohol, polyvinylpyrrolidone and polyoxyethylene oxypropylene glycol block copolymer.

The pH value of a transvaginal formulation for use in the present invention should preferably have a pH value close to that of the vagina, i.e., 3 to 7, preferably 4 to 6. The pH may be adjusted by an acid or base which is non-toxic and non-irritating to humans, for example, an organic acid such as acetic acid, or citric acid, or a weak base, such as sodium hydrogen carbonate, or sodium acetate.

A film preparation for use in the present invention may be prepared by mixing ILY with the above discussed liquid preparation, with a film base, for example, hydroxypropylmethyl cellulose, chitosan, pullulan, glucomannan, or polyacrylate ester.

A tampon-shaped preparation for use in the present invention may be prepared by coating a tampon-shaped core made of silicone resin with a polymer film containing ILY.

Pharmaceutical formulations suitable for vaginal administration, wherein the carrier is a solid, such as a wax, can be in the form of unit dose suppositories (contraceptive membrane suppositories). Suitable carriers include cocoa butter, agarose, dextran, glycerogelatin, or other materials commonly used in the art. The suppositories may be formed by admixture of ILY with the softened or melted carrier(s) followed by chilling and shaping in molds. Vaginal rings containing inert materials can also be used to introduce ILY into the female reproductive tract.

Additives that may be used in pharmaceutical compositions containing ILY may include excipients (such as starch, dextrin, mannitol, cyclodextrin and traganth) binding agents, fillers, colorants (such as beta-carotin), lubricants, isotonic agents (such as sodium chloride or glucose), disintegrants, antioxidants (such as ascorbic acid, erythorbic acid, or a salt or ester thereof), or wetting agents. Additional additives utilized in pharmaceutical compositions can be found, for example, in Remington: The Science and Practice of Pharmacy (Remington the Science and Practice of Pharmacy), 21st ed., (2005), edited by University of the Sciences in Philadelphia.

V. Animal models using cell ablation

The invention also features methods and compositions for killing hCD59 cells in an animal by administering ILY. Such methods and compositions are useful for studying the effects of ablation of certain cell types in development and physiology.

ILY rapidly and potently lyses human cells because it binds to the human complement regulator CD59 (hCD59). ILY does not bind hCD59 or lyse cells from any other animal species tested(6, 7). ILY kills cells by forming large-diameter (250-300Å), irreversible transmembrane pores. Oligomerization of up to 50 membrane-bound toxin monomers disrupts the membrane permeability barrier, causing cell lysis

within a few seconds. Based on this evidence, the invention features a method for ILY-mediated tissue specific cell ablation. We have demonstrated its effectiveness in two tissue-specific cell ablation transgenic mouse models, one expressing hCD59 only on erythrocytes (*ThCD59*^{RBC}) and the other only on endothelial cells (*ThCD59*^{END}). In *ThCD59*^{RBC}, the transgene was under the control of the alpha hemoglobin promoter and for *ThCD59*^{END} under the control of the ICAM-2 promoter (see experimental data set forth below). The invention features tissue specific expression of hCD59 by placing the hCD59 under other tissue specific promoters. An exemplary nucleic acid sequence encoding hCD59 is set forth in Table 1. Such promoters include, for example, the those set forth in Table 2.

Table 1 :hCD59 sequence

SEQ ID NO:	Gene	Sequence
1	hCD59	atgggaatcc aaggagggtc tgtcctgttc gggctgctgc tcgtcctggc tgtcttctgc cattcaggtc atagcctgca gtgctacaac tgtcctaacc caactgctga ctgcaaaaca gccgtcaatt gttcatctga ttttgatgcg tgtctcatta ccaaagctgg gttacaagtg tataacaagt gttggaagtt tgagcattgc aatttcaacg acgtcacaac ccgcttgagg gaaaatgagc taacgtacta ctgctgcaag aaggacctgt gtaactttaa cgaacagctt gaaaatggtg ggacatcctt atcagagaaa acagttcttc tgctggtgac tccatttctg gcagcagcct ggagccttca tccctaa

hCD59 can also be expressed in a cell-type or tissue specific manner through the use of a recombinase activation system. Such systems (for example the Cre-recombinase or FLP recombinase systems) contain a recombinase under the control of a tissue specific or cell-type specific promoter (see, for example, Buch, Nat Methods 2:419 (2005) and Saito et al., Nat Biotechnol 19:746 (2001)). When expressed in the specific tissue or cell type, this recombinase modifies the hCD59 transgene in the cellular genome causing hCD59 to be functionally expressed (for example, as described below). Such systems can be used, for example, to extend the use of this tissue specific cell ablation model for studies of cellular/tissue function, regeneration, and differentiation in almost any tissue (e.g., neural tissue, hematopoietic tissue, skin, endothelial tissue, and muscular tissue) or cell type. In one embodiment, the invention features combining the transgenic expression of hCD59 with the Cre-inducible system for a Cre-inducible hCD59 transgenic animal (e.g., a mouse). Through this strategy, the hCD59 mRNA is expressed in all cells within a transgenic animal, but the protein expression is blocked by a STOP codon, for example, placed

up-stream of the hCD59 open reading frame. After crossing the hCD59 transgenic animal with a transgenic Cre-recombinase expressing animal (e.g., mouse) that expresses the transgene in specific tissues of choice, the Cre-recombinase in that tissue will remove the STOP codon and allow the expression of hCD59 protein in the membrane of the specific cells selected. Examples of animals suitable for tissue specific expression of Cre recombinase and FLP recombinase are set forth in Table 2.

Specifically, a *Loxp*-flanked STOP cassette and the open reading frame of hCD59 gene is introduced into the *ROSA26* locus of the mouse by homologous recombination in embryonic stem cells to generate the knock-in transgenic mouse (*LoxP-STOP-LoxP-hCD59*$^{+/-}$). Upon injection of ILY, those cells carrying transgenic hCD59 protein will be targeted by ILY and acutely destroyed thus creating a cell/tissue specific ablation model for that cell/tissue.

Table 2. Mouse models for tissue specific gene expression

STOCK NUMBER / STRAIN NAME	PROMOTER (SPECIES)	SITE OF EXPRESSION & COMMENTS
Cre Cell Specific Expression		
005936 STOCK Tg(ACTA1-cre)79Jme/J	ACTA1, actin, alpha 1, skeletal muscle (human)	adult striated muscle fibers and embryonic striated muscle cells of the somites and heart
004302 129S1-Hprt1tm1(cre)Mnn/J	Actb, actin, beta, cytoplasmic (mouse)	widespread pattern of expression; transgenic and wildtype oocytes both confer *cre* activity
003376 FVB/N-Tg(ACTB-cre)2Mrt/J	ACTB, actin, beta (human)	all cells of the embryo by the blastocyst stage of development; widespread expression
003574 B6.Cg-Tg(Alb-cre)21Mgn/J	Alb, albumin (rat)	Liver
005359 B6.Cg-Tg(Camk2a-cre)T29-1Stl/J	Camk2a, calcium/calmodulin-dependent protein kinase II alpha (mouse)	forebrain; pyramidal cell layer
005735 NOD.Cg-Tg(CD4-cre)1Cwi/AchJ	CD4, CD4 molecule (human)	T cells
004126 C.129P2-Cd19tm1(cre)Cgn/J	Cd19, CD19 antigen (mouse)	B cells
004426 B6;SJL-Tg(Cga-cre)3Sac/J	Cga, glycoprotein hormone alpha subunit (mouse)	anterior and intermediate lobes of the pituitary gland, as well as in cardiac and skeletal muscle; low to no level of expression is detected in the posterior pituitary, lungs, kidneys, brain, adrenal gland and gonads
005105 STOCK Tg(Chx10-EGFP/cre-ALPP)2Clc/J	Chx10, C. elegans ceh-10 homeo domain containing homolog (mouse)	expression pattern is mosaic, but specific to the retina and Muller glial cells
003465 BALB/c-Tg(CMV-cre)1Cgn/J 002471 STOCK Tg(hCMV-cre)140Sau/J	CMV, cytomegalovirus (human)	widespread expression, including germline
003554 B6;SJL-Tg(Col2a1-cre)1Bhr/J	Col2a1, procollagen, type II, alpha 1(mouse)	differentiating chondrocytes, notochord, submandibular glands
003724 B6.FVB-Tg(EIIa-cre)C5379Lmgd/J 003314 FVB/N-Tg(EIIa-cre)C5379Lmgd/J	EIIa, adenovirus	widespread expression (including germline); mosaic pattern commonly observed
005628 B6.129-Emx1tm1(cre)Krj/J	Emx1, empty spiracles homolog 1 (Drosophila)	neurons of the neocortex and hippocampus
005069 B6.Cg-Tg(Fabp4-cre)1Rev/J	Fabp4, fatty acid binding protein 4, adipocyte (mouse)	adipose tissue

004337 129.Cg-Foxg1tm1(cre)Skm/J	Foxg1, forkhead box G1 (mouse)	telencephalon, anterior optic vesicle (developing lens and retina), otic vesicle, facial and head ectoderm, olfactory epithelium, mid-hindbrain junction and pharyngeal pouches
004600 FVB-Tg(GFAP-cre)25Mes/J	GFAP, glial fibrillary acidic protein (human)	primarily in the central nervous system, affecting astrocytes, oligodendroglia, ependyma and some ,neurons; also periportal cells of the liver
003734 B6;FVB-Tg(GZMB-cre)1Jcb/J	GZMB, granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1) (human)	activated T cells
004692 STOCK Tg(Hoxb7-cre)13Amc/J	Hoxb7, homeo box B7 (mouse)	mesonephric duct and its developmental derivatives (the Wolffian duct, the collecting duct epithelium of kidney and ureteral epithelium), ureteric bud and all ureteric bud epithelial cells; low levels of expression in the dorsal root ganglia and the spinal cord
003573 B6.Cg-Tg(Ins2-cre)25Mgn/J	Ins2, insulin 2 (rat)	pancreatic beta cells
004782 STOCK Tg(KRT14-cre)1Amc/J	KRT14, keratin 14 (human)	skin, the oral ectoderm including the dental lamina at 11.75 d.p.c., and the dental epithelium by 14.5 d.p.c
003802 B6.Cg-Tg(Lck-cre)548Jxm/J 005732 NOD.Cg-Tg(Lck-cre)548Jxm/AchJ	Lck, lymphocyte protein tyrosine kinase (mouse)	Thymocytes
004781 B6.129P2-Lyzstm1(cre)Ifo/J	Lyzs, lysozyme (mouse)	myeloid cells - including monocytes, mature macrophages, and granulocytes
003755 B6.129S4-Meox2tm1(cre)Sor/J	Meox2, mesenchyme homeobox 2 (mouse)	epiblast-derived tissues as early as embryonic day 5; all primitive ectoderm by embryonic day 7 (but not endoderm or trophectoderm); null allele for the Meox2 gene
003551 STOCK Tg(MMTV-cre)1Mam/J 003553 STOCK Tg(MMTV-cre)4Mam/J	MMTV, Mouse Mammary Tumor Virus widespread pattern of expression	including the female germline; high levels of recombination have been detected in the virgin and lactating mammary gland, salivary gland, seminal vesicle, and various cells of the immune system; little background recombination was observed in the lung, kidney, liver and brain tissues
003771 B6.Cg-Tg(Nes-cre)1Kln/J	Nes, nestin (rat)	primarily expressed in the central and peripheral nervous system by embryonic day 11 and a few isolated kidney and heart cells
002858 STOCK Tg(Nes-cre)1Wme/J	Nes, nestin (rat)	induces variable mosaicism/partial deletion of loxP-flanked alleles before embryonic day 10.5 and in all adult organs (including germline), producing mosaic mice consisting of cells that are either wildtype or mutant for the gene of interest
002859 STOCK Tg(Nes-cre)2Wme/J	Nes, nestin (rat)	induces variable mosaicism/partial deletion of loxP-flanked alleles before embryonic day 10.5 and in all adult organs (including germline), producing mosaic mice consisting of cells that are either wildtype or mutant for the gene of interest, more variable mosaicism than the "balancer 1" line (Stock No. 002858)
005667 STOCK Tg(Neurog3-cre)C1Able/J	Neurog3, neurogenin 3 (mouse)	small intestine (base of intestinal crypts) and fetal pancreatic epithelial cells
005697 B6.129-Otx1tm4(cre)Asim/J	Otx1, orthodenticle homolog 1 (Drosophila)	lateral midbrain of embryonic day 10.5 aged embryos and in the presumptive alar-basal plate boundary of embryonic day 12.5 aged embryos

005549 B6;129-Pax3tm1(cre)Joe/J	Pax3, paired box gene 3 (mouse)	dorsal neural tube and somites of embryonic day 9-11.5 embryos and in the cardiac neural crest cells and colonic epithelia of embryonic day 11.5 embryos
004146 B6.129-Tg(Pcp2-cre)2Mpin/J	Pcp2, Purkinje cell protein 2 (L7) (mouse)	most Purkinje cells and some retinal bipolar neurons; small amounts of activity are observed in an unidentified population of cells of the central nervous system tissue; recombination is first observed around postnatal day 6 and is fully established 2 to 3 weeks after birth
003328 129-Tg(Prm-cre)58Og/J	Prm1, protamine 1 (mouse)	male germ line
003960 129S6-Tg(Prnp-GFP/cre)1Blw/J	Prnp, prion protein (mouse)	widespread expression (including germline) as early as the 4-8 cell embryonic stage; GFP expression is less robust
005584 B6.Cg-Tg(Prrx1-cre)1Cjt/J	Prrx1, paired related homeobox 1 (rat)	early limb bud mesenchyme and in a subset of craniofacial mesenchyme, some female germline expression
005989 129;FVB-Tg(PTH-cre)4167Slib/J	PTH, parathyroid hormone (human)	parathyroid tissue
003967 B6.Cg-Tg(Rbp3-cre)528Jxm/J	Rbp3, retinol binding protein 3, interstitial (rat)	photoreceptor cells
004783 STOCK Tg(Sox2-cre)1Amc/J	Sox2, SRY-box containing gene 2 (mouse)	epiblast cells at embryonic day 6.5, with little or no activity in other cells at gastrulation. Some activity is also detected in extra embryonic derivatives of the epi-blast, the yolk sac mesoderm and amnion. No activity is detected in primitive endoderm derived tissues, visceral endoderm
003466 B6;D2-Tg(Sycp1-cre)4Min/J	Sycp1, synaptonemal complex protein 1 (mouse)	male germ cells; expressed at the leptotene-zygotene stage of male meiosis
003966 B6.Cg-Tg(Syn1-cre)671Jxm/J	Syn1, synapsin I (rat)	neuronal cells by embryonic day 12.5
004746 STOCK Tg(Tagln-cre)1Her/J	Tagln, transgelin (mouse)	vascular smooth muscle cells
004128 B6.Cg-Tg(Tek-cre)12Flv/J	Tek, endothelial-specific receptor tyrosine kinase (mouse)	female germline as well as tyrosine endothelial and hematopoietic cells; a low frequency of deletion events are also observed by inheritance from the male germline
006143 FVB/N-Tg(Thy1-cre)1Vln/J	Thy1, thymus cell antigen 1, theta (mouse)	neurons of the postnatal cortex and hippocampus
004586 B6.SJL-Tg(Vil-cre)997Gum/J	Vil1, villin 1 (mouse)	epithelial cells of the small and large intestines; onset of expression is at 12.5 d.p.c.; expression is generally continuous, but a small amount of mosaicism is noted in the colon
003552 B6129-Tg(Wap-cre)11738Mam/J	Wap, whey acidic protein (mouse)	mammary gland tissues, specifically the secretory epithelium; not entirely restricted to mammary gland, a limited amount of cre activity has been reported in brain tissue
003829 STOCK Tg(Wnt1-GAL4)11Rth	Tg(Wnt1-cre)11Rth/J	Wnt1, wingless-related MMTV integration site 1 (mouse) embryonic neural tube, midbrain, dorsal and ventral midlines of the midbrain and caudal diencephalon, the mid-hindbrain junction and dorsal spinal cord
003394 C57BL/6-Tg(Zp3-cre)3Mrt/J 003377 FVB/N-Tg(Zp3-cre)3Mrt/J	Zp3, zona pellucida glycoprotein 3 (mouse)	female germ line; growing oocyte prior to first meiotic division
003651 C57BL/6-Tg(Zp3-cre)93Knw/J	Zp3, zona pellucida glycoprotein 3 (mouse)	female germ line; growing oocyte to the completion of the first prior meiotic division

Cre-recombinase Expression Inducible strains		
004682 B6.Cg-Tg(cre/Esr1)5Amc/J 004453 STOCK Tg(cre/Esr1)5Amc/J	ACTB, actin, beta (chicken)	tamoxifen-inducible cre; widespread pattern of expression; tamoxifen administration will also induce cre recombination in developing embryos of treated mothers and in cultured cells derived from transgenic mice
004192 STOCK Mttptm2Sgy Ldlrtm1Her Apobtm2Sgy Tg(Mx1-cre)1Cgn/J	cre: Mx1, myxovirus (influenza virus) resistance 1 (mouse) interferon alpha-, interferon beta-, or synthetic double-stranded RNAinducible loxP: Mttp, microsomal triglyceride	cre mutation deletes floxed Mttp transfer protein (mouse) gene, reversing hypercholesterolemia phenotype
004847 B6;129-Gt(ROSA)26Sortm1(cre/Esr1)Nat/J	Gt(ROSA)26Sor, gene trap ROSA 26, Philippe Soriano (mouse)	tamoxifen-inducible cre; when crossed with a strain containing a loxP siteflanked sequence of interest, this mutant is useful for generating tamoxifen-induced, cre-mediated targeted deletions
005107 STOCK Tg(KRT14-cre/Esr1)20Efu/J	KRT14, keratin 14 (epidermolysis bullosa when crossed with a strain containing simplex, Dowling-Meara, Koebner) (human)	a loxP site flanked sequence of interest, the offspring are useful for generating tamoxifen-induced, cre mediated targeted keratinocyte specific deletions
005249 B6;SJL-Tg(Krt1-15-cre/PGR)22Cot/J	Krt1-15, keratin complex 1, acidic, gene 15 (mouse)	when crossed with a strain containing a loxP site flanked sequence of interest, the offspring are useful for generating RU 486-induced, cre-mediated targeted deletions
005650 B6129-Tg(Myh6-cre/Esr1)1Jmk/J	Myh6, myosin, heavy polypeptide 6, cardiac muscle, alpha, murine	Tamoxifen inducible (yet estrogen insensitive) Cre recombinase protein fused to two mutant estrogen-receptor ligand-binding domains (MerCreMer); expression in developing and adult heart
003556 B6.Cg-Tg(Mx1-cre)1Cgn/J 005673 C.Cg-Tg(Mx1-cre)1Cgn/J 002527 STOCK Tg(Mx1-cre)1Cgn/J	Mx1, myxovirus (influenza virus) resistance 1 (mouse)	widespread pattern of expression; promoter induced to high levels of transcription by administration of interferon alpha, interferon beta, or synthetic double-stranded RNA; provides capability to induce the "knockout" at any time during development
005975 B6.Cg-Tg(Plp1-cre/Esr1)3.16Pop/J	Plp1, proteolipid protein (myelin) 1 (mouse)	tamoxifen inducible Cre recombinase protein fused to mutant form of the mouse estrogen receptor ligand binding domain; expression in oligodendrocytes and Schwann cells
005623 B6.129S-Shhtm2(cre/ESR1)Cjt/J	Shh, sonic hedgehog (mouse)	tamoxifen inducible Cre recombinase protein fused to a mutant form of the human estrogen receptor ligand binding domain; expression in all cells that express the Shh gene

VI. Experimental Results

Streptococcus intermedius (SI) is part of the normal human oral micro flora and can cause liver and brain abscesses. ILY secreted by SI specifically binds and lyses CD59 positive human cells. Our results demonstrate that human serum, but not the serum from any other tested species, can neutralize the lytic function of ILY. We have identified an ILY-binding human immunoglobulin (IgG) purified from human serum that exhibits a functional inhibitory effect on ILY-mediated hemolysis *ex vivo* and *in vivo*.

Using FACS analysis with anti-CD59 antibodies and RT-PCR, hCD59 expression has been detected on the surface of adipocytes at both the protein and mRNA level. hCD59 is also highly expressed in sperm cells and has been suspected to play an important role in male fertility. Deficiencies in mouse hCD59 expression results in the progressive loss of male fertility, suggesting a role of hCD59 in male reproduction.

CD59 is also highly expressed in various cancer cells such as prostate, breast and gastric adenomas and intestinal-type gastric carcinomas, and B-cell lymphoma. Higher expression levels of hCD59 in neoplastic cells has been correlated with cellular resistance to certain chemotherapeutic drugs. We have demonstrated that ILY specifically binds to human CD59 and lyses human cells that express human CD59 on the cell surface. We found that humans develop specific immunity to protect cells from ILY-mediated cell lysis.

1. Experimental model

We used a plasmid containing His-tagged ILY to express and purify recombinant ILY from bacteria using a His Bind Purification Kit (Novagen). In order to show that hCD59 is the only receptor for ILY in human RBC, we have performed *ex vivo* and *in vivo* experiments with *hCD59RBC*.

For the *ex vivo* study, we demonstrated that the expression of hCD59 in the RBC of *hCD59RBC* transgenic mice makes the *hCD59RBC*$^{+/-}$ mRBCs hyper-sensitive to ILY-mediated lysis. This lysis is comparable to the level of ILY-mediated lysis of human RBC (Fig. 1A). WT mRBC are resistant to ILY-mediated lysis. For the *in vivo* study, we administrated different doses of ILY by tail vein injection. With three different doses of ILY (95, 47, 30 ng/g body weight), the percentages of ILY-induced cell death of *hCD59RBC* transgenic mice were 100% (15/15 animals), 50% (8/16 animals) and 0% (0/6 animals), respectively. Based on this data, we consider the dose of 95 ng ILY/g body weight as the lethal dose (LD_{100}) in our *in vivo* experiments.

There was no observed cell death in RBCs from WT mice (0/6 animals), even at the highest levels of treatment (3000ng ILY/g body weight of ILY). Injecting 45 ng ILY/g body weight induced massive hemolysis in *hCD59RBC*$^{+/-}$, but not in WT mice, as demonstrated by decreased the hematocrit values and visible hemolysis and hemoglobinuria. Fig. 1B shows significantly reduced hematocrit values in

hCD59RBC$^{+/-}$ as compared to WT mice at 10 minutes, 1 day, and 4 day after ILY injection. Visible hemolysis was found in five *hCD59RBC*$^{+/-}$ mice, but not in five WT mice at 10 minutes after ILY injection (Fig. 1C). Visible hemogloabinuria was found in the urine from *hCD59RBC*$^{+/-}$ but not in WT mice, when collected at 5 hours and 1 day after ILY injection (Fig. 1D).

2. Inhibitors in human serum neutralize the lytic effect of ILY.

Because SI (1) is part of the normal human oral micro flora, (2) can cause liver and brain abscesses, and (3) secretes ILY that specifically lyses human cells due to the presence of hCD59 in their surface, we propose that humans may develop some immune defenses that protect against ILY-mediated cell lysis and pathogenic SI infection. In order to test this hypothesis, we performed hemolytic assays with human RBC. Human RBC were incubated with different concentrations of ILY and a 1 to 8 dilution of serum in PBS from different species. Fig. 2A shows that human serum, and not the serum from other 11 animal species, significantly blocked ILY-mediated human RBC lysis.

In order to isolate the inhibitors from human serum, we used an ILY-binding sepharose column. By hemolytic assay, we verified that the eluted fraction and not the flow through of human serum has functional activity that protects human RBC from ILY-mediated hemolysis (Fig. 2B). The specific proteins that bound to the ILY were separated on SDS-PAGE gel (Fig. 2C) and were isolated for protein sequencing. We found IgG in the ILY-eluted fractions of human and mouse serum. MAC-2BP protein was only found in the ILY-eluted fraction of human serum (Fig. 2D).

3. Isolation of ILY inhibitors and their protective effect.

In order to separate the human IgG or mouse IgG from MAC-2BP and SP40, we further purified the eluted fraction from the ILY-binding column with a protein G column, which binds to IgG (Fig. 3B). We used a hemolytic assay to test the functional activity of the eluted fractions from human serum purified on the ILY column and the protein G column. Fig. 3B shows the eluted fraction from the protein G column has a functional activity similar to that of the eluted fraction from the ILY column. We have purified approximately 300 μg of ILY-binding human IgG or mouse IgG from 1 ml of each species' serum. Normally, there is an average of 12

mg/ml of total human IgG in human serum. Therefore, 2.5% of the total human IgG can bind to ILY. Only the ILY-binding IgG from human serum, and not from mouse serum, has functional activity in protecting hRBC from ILY-mediated hemolysis (Fig. 3B). ILY-binding mouse IgG does not have any protective activity against ILY-mediated lysis (Fig. 3C).

A possible explanation for why the 2.5% of mouse IgG that specifically binds to ILY does not block ILY mediated cell lysis follows. First, SI is part of the normal micro flora only in humans, not in the mouse and other animals. Thus, the human immune system can be exposed to ILY and produce antibodies specific to the functional domain of ILY, including the domain 4 (a binding site for hCD59). Second, there may be cross-reactivity of ILY with antibodies against the cytolytic toxins that are produced by other Gram-positive bacteria, including species within the genera *Clostridium*, *Streptococcus*, *Listeria,* and *Bacillus*, which are normal microflora in humans and other animals. The members of this toxin family-including ILY-exhibit 40-80% similarity at the primary sequence level. Humans and other animals can produce antibodies specific for a variety of these toxins. It is possible that these antibodies can cross react with different regions of ILY, but not neutralize ILY function.

In order to further determine which part of this ILY-binding human IgG molecule binds to ILY, 200 ng ILY was used to coat wells in a 96-well plate followed by incubation with serial dilutions of the eluate from human serum loaded on the ILY-binding column. Saturating amounts of secondary antibodies (goat anti-human IgG Fab or Fc fragment-HRP antibodies) were used to detect free binding sites in the wells after the ILY-coated wells were pre-incubated with serial dilutions of human serum eluate. Figure 3D shows significantly more-free binding site for anti-human Fc secondary antibody than for anti-human Fab secondary antibody. This result suggests that the site where the ILY-binding human IgG binds to ILY may reside in the Fab region. Furthermore, using ELISA, we demonstrated that ILY does not bind to the Fc region of human IgG. In this method ILY was used to coat wells in a 96-well plate, the wells were incubated with different concentrations of commercial human Fc fragments, and Fc fragment binding was detected using an anti-human Fc fragment-HRP antibody. This result further supports the conclusion that ILY-binding human IgG binds to ILY by the Fab region.

In order to determine the *in vivo* effect of ILY-binding human IgG, we first treated *hCD59RBC*$^{+/-}$ with an intravenous (IV) injection of different doses of the ILY-binding human IgG. Fifteen minutes later, we injected these mice with 285 ng ILY/g body weight (three times lethal dose (LD) of ILY in untreated *hCD59RBC*$^{+/-}$ mice). We found that the survival percentage of *hCD59RBC*$^{+/-}$ pre-treated with 1 and 0.75 μg ILY-binding human IgG/g body weight was 89% (8/9) and 62.5% (5/8), respectively. This result confirms that the ILY-binding human IgG blocks ILY function *in vivo* and suggests that an anti-ILY antibody may be useful for the treatment of SI infectious disease.

4. Tissue specific cell ablation

As described above, the methods of the invention are useful for tissue specific cell ablation in animal models of development and disease. The below example demonstrates several working embodiments of tissue specific cell ablation using expression of hCD59 and ILY.

Transgenic mice express human CD59 on erythrocytes and endothelia

CD59 is a membrane-bound complement regulator that inhibits formation of the membrane attack complex (Qin Immunity 18:217–227 (2003)). We generated ThCD59^{RBC} mice expressing human CD59 in erythrocytes (Kooyman et al. Science 269:89–92 (1995)); human CD59 expression on erythrocytes in these mice was associated with increased resistance to membrane attack complex–mediated lysis, and expression of human CD59 mRNA was specific to hematopoietic organs (Figs. 9A-C). We also generated ThCD59^{END} mice expressing human CD59 on endothelia; human CD59 mRNA and protein was expressed only in blood vessels in these mice (Figs. 9D and 9E). The response to ILY administration *in vivo* and *ex vivo* was similar in six ThCD59^{RBC} lines and in three ThCD59^{END} lines, so we used one heterozygous ThCD59^{RBC} line and one heterozygous ThCD59^{END} line for further experiments.

Rapid conditional targeted erythrocyte ablation in ThCD59^{RBC}

Human and ThCD59^{RBC} erythrocytes were markedly more sensitive to ILY-mediated lysis (by factors of 2.0 x 10^5 and 4.8 x 10^4, respectively) than wild-type erythrocytes (Figs. 4A and 4B). The highest practical ILY concentration (24 μM) lysed approximately 32% of wild-type erythrocytes, whereas 125 pmol/L. and 500 pmol/L. were sufficient to lyse a similar percentage of human or ThCD59^{RBC} erythrocytes, respectively (Fig. 4B).

In vivo, ILY injection induced rapid dose-dependent intravascular hemolysis in ThCD59^{RBC} mice but not in wild-type mice, as indicated by reduced hematocrit values (Fig. 4C and 4D) associated with increased plasma hemoglobin (Fig. 4E) and marked hemoglobinuria (Fig. 4F) in ThCD59^{RBC} mice. Injection of 60 ng ILY per g body weight caused sudden death associated with massive hemolysis within 1 min in 50% of the ThCD59^{RBC} mice (LD_{50}). In contrast, injection of up to 3,000 ng per g body weight did not cause any death in wild-type mice (n = 6).

Only humans produce specific ILY-neutralizing antibodies

To investigate whether human cells are protected by antibodies against ILY-mediated lysis, we showed that the dose of ILY required to lyse 50% of human erythrocytes is at least 35 times higher in the presence of human serum than in the presence of sera from 11 other species (Fig. 5A and Table 3 below).

Table 3. Dose of ILY (x 10^{-10} M) required to lyse 50% of human erythrocytes after pre-incubation with sera from different species diluted 1 to 8 in PBS

ILY (no serum)	Human	Dog	Cow	Pig	Guinea Pig	Horse	Goat	Rabbit	Monkey	Rat	Mouse	Sheep
0.53	116.67	0.17	0.42	3.33	0.25	0.42	0.42	1.50	1.50	2.17	2.50	0.45

We isolated the inhibitor of ILY-mediated lysis in human serum using an ILY-conjugated Sepharose affinity column. We identified the affinity-bound fraction retaining the protective activity (Fig. 5A) as a major band in SDS-PAGE and further purified it with a protein G affinity column (Fig. 5B). The fraction eluted from the protein G affinity column showed a protective potency similar to the fraction eluted

by the ILY affinity column (Fig. 5D). Sequencing of the major band (Fig. 5C, lane 9) showed that the protein is an IgG, further subtypified as IgG1, IgG2, IgG3 and IgG4.

We also isolated an ILY-binding IgG from mouse and bovine sera (confirmed by sequencing) (Fig. 5C), but this ILY-binding IgG did not show any ILY-neutralizing activity (Fig. 5E).

ILY-neutralizing human IgG prevents erythrocyte ablation

Pretreatment of ThCD59^{RBC} mice with ILY-neutralizing human IgG prevented death after administration of a lethal dose of ILY, but pretreatment with ILY-binding bovine IgG did not have this preventive effect (Fig. 6A). ILY-mediated intravascular hemolysis in ThCD59^{RBC} mice was also prevented by human IgG but not by bovine IgG (Fig. 6B).

During intravascular hemolysis, increased cell-free hemoglobin scavenges endothelium-derived nitric oxide (NO) and disrupts NO homeostasis, leading to platelet activation, adhesion, and aggregation. Consistently, ILY injection in ThCD59^{RBC} mice markedly decreased NO levels and increased platelet activation, as shown by increased plasma P selectin (sP-selectin)—an effect prevented by pretreatment with ILY-neutralizing human IgG (Figs. 6C and 6D).

Rapid conditional targeted endothelial ablation in ThCD59^{END}

We confirmed successful ILY-mediated endothelial ablation in ThCD59^{END} mice (but not in wild-type mice) by a significantly higher number of circulating endothelial cells (Figs. 7A and 7B), extensive Evans blue aortic staining (Fig. 7C), ultrastructural changes characteristic of endothelial damage (Figs. 7D-7F) and significant increases in von Willebrand factor (vWF) (Fig. 7G) in ThCD59^{END} mice. As would be consistent with massive endothelial damage, all ThCD59^{END} mice (but not wild-type mice) died rapidly after a lethal dose of ILY. All of these effects were prevented by pretreatment with ILY-neutralizing human IgG (Fig. 7H).

We also used ThCD59^{END} mice to examine the sequelae of nonlethal ILY-mediated endothelial damage. Multiple injections of ILY to ThCD59^{END} mice induced clinical manifestations characteristic of disseminated intravascular coagulation: (i) severe lung tissue damage with intra-alveolar edema, neutrophil margination, perivascular inflammation with neutrophil infiltration and thrombosis

(Fig. 8A); (ii) enhanced activation of coagulation cascades, as demonstrated by increases in the sensitive intravascular coagulation marker thrombin-antithrombin III complex (TAT; Fig. 8B) and (iii) increased platelet consumption, evidenced by significantly reduced platelet counts (Fig. 8C).

We report the use of ThCD59^{RBC} and ThCD59^{END} mice for the prototypic development of a new model of rapid conditional targeted cell ablation that takes advantage of ILY-mediated lysis exclusively on cells carrying human CD59. Transgenic mice carrying human CD59 helped confirm *in vivo* that human CD59 is the only receptor for ILY. This ILY-mediated cell ablation system may be useful for cellular function and/or tissue regeneration studies not only in mice but also in intermediate-sized and large animals transgenically expressing human CD59 in the cells or organs of interest.

The erythrocytic and endothelial ablation models established here provide a new tool for studying the pathogenesis of and potential treatments for human diseases characterized by hemolytic anemia or endothelial damage. In ThCD59^{RBC} mice, ILY mediates intravascular hemolysis, a common manifestation of several human diseases, including paroxysmal nocturnal hemoglobinuria, sickle cell disease, autoimmune anemias, thalassemias, transfusion reactions and infection-induced anemias. The intravascular hemolysis caused by these conditions results in common clinical manifestations such as abdominal pain, dysphagia, erectile dysfunction, endothelial dysfunction, pulmonary hypertension, renal failure and platelet activation with thrombosis. The NO scavenger effect of free plasma hemoglobin is suspected to be the underlying mechanism of these complications, known as sequelae of intravascular hemolysis.

In ThCD59^{END} mice, ILY mediates severe endothelial damage; this may serve as a model for the study the pathogenesis of cardiovascular diseases. Extensive experimental evidence indicates that the functional integrity of the endothelium is critical for many important homeostatic and cellular functions and that early endothelial damage contributes to the increased cell adhesion and inflammation seen in atherosclerosis, ischemia-reperfusion injury, acute vasculitis, autoimmune disease and disseminated intravascular coagulation.

The broad use of ILY-mediated targeted cell ablation not only in mice but also in other animal species is made possible by specific features that we have documented here: (i) ILY does not damage cells from any animal species other than humans, (ii) ILY shows high affinity and specificity for human CD59 *in vivo*, allowing the ablation of specific cell populations by targeted expression of transgenic human CD59, and (iii) ILY-neutralizing antibodies are absent in species other than humans, allowing the use of ILY in transgenic animals.

Why ILY-neutralizing antibodies are present only in humans is still unclear. One possible explanation is that humans (but not other species) are routinely exposed to infection with *Streptococcus intermedius* and ILY and have developed specific antibodies against functional domain(s) of ILY—in particular, domain 4, which serves as the binding site for human CD59. The presence of non-neutralizing ILY antibodies in other species may be explained by ILY belonging to the CDC family of toxins, which contributes to the pathogenesis of a variety of human and animal diseases caused by Gram-positive bacteria. The 40–80% sequence similarity between ILY and more than 20 members of the CDC family is probably responsible for the antibody cross-reactivity. CDC binding antibodies may bind to ILY but not to ILY domain 4, which is critical for the binding of ILY to human CD59 and for lytic activity.

We have shown that repetitive sublethal ILY doses can be used to induce progressive and sustained ablation, opening the possibility of conducting long-term experiments. For this approach, it will be important to consider that although sera from nonhuman species do not contain ILY-neutralizing antibodies, these species may develop specific IgGs that neutralize ILY function after multiple long-term injections. If this were the case, gradually increased ILY doses might be needed in chronic experiments with human CD59 transgenic animals to compensate for any diminished efficacy of ILY due to induced immune responses.

In addition to its possible use in a broad range of species transgenically expressing human CD59, the ILY-mediated cell ablation model has other attractive features. First, the effective cell ablation occurs very rapidly—within seconds after ILY administration. In contrast, diphtheria toxin–mediated cell damage in *Hbegf* transgenic mice is effective within a time frame of days. ILY-mediated rapid cell ablation may help define primary pathophysiological changes without confounding effects due to adaptive responses of other cells. Second, ILY-mediated cell ablation is

specifically confined to cells carrying human CD59. ILY lyses only human cells, although cells from other species also carry homologous CD59. In the prototypic models, mouse erythrocytes transgenically expressing human CD59 became about 50,000 times more sensitive to ILY-mediated lysis than wild-type erythrocytes. Third, ILY-mediated cell ablation is highly efficient and potent. At doses as low as 30 ng per g body weight and 4.5 ng per g body weight, ILY induces massive intravascular hemolysis in ThCD59^{RBC} mice and severe endothelial damage in ThCD59^{END} mice, respectively, without any evidence of off-target effects in wild-type mice or in transgenic mice pretreated with neutralizing antibodies. Fourth, ILY-mediated cell ablation can be achieved by several routes of administration: intravenous, intraperitoneal and intramuscular administration all produce comparable results (Figs. 10A and 10B). Furthermore, ILY can pass through the endothelial barrier. After ILY injection, ILY is engulfed by resident macrophages in organs such as brain, liver and lung, as shown by double immunofluorescence assay (Figs. 11A-11C). Fifth, recombinant ILY can be easily procured from prokaryotic expression systems. Finally, ILY-mediated cell ablation may be the method of choice for ablation of nondividing cells such as erythrocytes.

In summary, the ILY-mediated *in vivo* specific cell ablation method reported here represents an alternative model applicable to studies of cell function, tissue regeneration and differentiation and will be useful for investigating the pathogenesis of and potential therapies for prevalent human ailments such as hemolytic anemia and cardiovascular diseases.

Methods

Preparation of recombinant ILY. We purified His-tagged recombinant ILY as described previously (Giddings et al. Nat. Struct. Mol. Biol. 11:1173–1178 (2004)).

Hemolytic assay. We performed hemolytic assays as described previously (Giddings et al. Nat. Struct. Mol. Biol. 11:1173–1178 (2004)). To evaluate a possible protective effect against ILY-mediated hemolysis of red blood cells, we added serially diluted ILY to human erythrocytes preincubated with human serum at a 1:8 dilution in PBS (ComTech). We tested sera from other species (Valley Biomedical) for ILY inhibitory effects by a hemolytic assay. Before the hemolytic assay, the eluate and

flow-through fractions were dialyzed and concentrated to volumes equal to the volume of serum that was loaded on the column.

Determination of ILY LD_{50} in $ThCD59^{RBC}$ mice. We randomly assigned eight mice per group (four males and four females) to one of five groups and recorded the percentage of mice surviving or dying within 6 h after intravenous (i.v.) ILY injection.

Hematocrit, plasma hemoglobin and hemoglobinuria measurement. We measured hematocrit, plasma hemoglobin and hemoglobinuria as described previously (Qin Immunity 18:217–227 (2003)).

Measurement of nitric oxide products nitrite and nitrate. We measured the concentration of the NO products nitrite and nitrate 1 h after ILY treatment, using a Nitric Oxide Quantitation Kit (ActiveMotif).

sP-selectin measurement. We collected mouse blood samples by venipuncture from the inferior vena cava with a syringe containing 10 mM EDTA and centrifuged samples (2,000*g*, 20 min) 1 h after ILY treatment. We then centrifuged the plasma supernatant (10,000*g*, 10 min) and assayed sP-selectin in the platelet-free plasma by ELISA (R&D Systems).

Evans blue dye staining. We examined endothelial cell damage by Evans blue staining, a method for evaluating endothelial damage (Brouchet et al. Circulation 103:423–428 (2001)). Briefly, we pretreated all the mice with either PBS or ILY-binding human IgG (1 μg per g body weight, i.v.) and injected them with ILY (4.5 ng per g body weight, i.v.) 15 min later. We injected 50 μl of solution containing 5% Evans blue dye (Sigma-Aldrich) in PBS into the tail vein 2 h after ILY treatment. We killed the mice 10 min after Evans blue dye injection and then fixed them by perfusion with 4% phosphate-buffered formalin (pH 7). Next, we harvested mouse aortas, opened them along the long axis and evaluated blue-stained areas by light microscopy. We quantified Evans blue staining areas using Image ProPlus 6.0.

von Willebrand Factor (vWF) measurement. vWF is a biomarker for endothelial damage. We measured mouse plasma vWF by ELISA as described previously (Denis et al. Proc. Natl. Acad. Sci. USA 95:9524–9529 (1998)). We pretreated mice with either PBS or ILY-neutralizing human IgG (1 μg per g body weight) for 15 min, injected them with ILY (3 ng per g body weight) and then measured plasma vWF 3 h after ILY injection.

Electron microscopy. We pretreated all mice with either PBS or ILY-binding human IgG (1 μg per g body weight, i.v.). We then injected one dose of ILY (4.5 ng per g body weight, i.v.) 15 min later and injected a second dose 6 h later. We killed the mice 6 h after the second ILY injection and prepared tissues for ultrastructural analysis, histological analysis, TAT measurement and platelet count, as described below.

We fixed tissues with 2.5% glutaraldehyde, postfixed them with osmium, stained them *en bloc* with 1.5% uranyl acetate, dehydrated them in ethanol and embedded them in Epon. We contrasted ultrathin sections with uranyl acetate and Reynolds lead citrate solution and examined them with a Jeol JEM-20 CX electron microscope. We purchased all reagents from Electron Microscopy Science.

TAT measurement. TAT, a stable complex formed by the reaction of thrombin with its major inhibitor antithrombin III, represents a sensitive marker for the activation of intravascular coagulation,. We measured TAT using an ELISA kit (Behring Diagnostic).

Platelet count. We counted platelets as described previously (Qin Immunity 18:217–227 (2003)).

Histological analysis of tissue damage. We fixed tissues in Bouin's solution, embedded 5-mm slices in paraffin and stained 6-μm sections with hematoxylin and eosin. We analyzed histological sections obtained from fixed, sectioned tissues using a Nikon Eclipse TE2,000-E microscope equipped with a Spot digital camera.

Statistical analysis. We determined the statistical differences between two groups by one-tailed Student's *t* test for unpaired data. To compare values obtained from three or more groups, we used one-factor analysis of variance (ANOVA) followed by Tukey's *post hoc* test. We used $P < 0.05$ as the threshold for statistical significance. We calculated the LD_{50} for the amount of ILY injection according to the Dixon-Mood method (Dixon et al. J. Am. Stat. Assoc. 43:109–126 (1948)).

Generation of ThCD59^{RBC} transgenic mice. We generated ThCD59^{RBC} mice expressing hCD59 in erythrocytes in C57BL/BJ background with the construct described previously (Saito et al. Nat. Biotechnol. 19:746–750 (2001)). The transgenic vector consisted of the human alpha hemoglobin locus control region, alpha hemoglobin gene promoter, and *hCD59* cDNA (from 5' to 3'). Non-transgenic C57BL/6J mice were WT controls.

Generation of ThCD59^{END} transgenic mice. We generated ThCD59^{END} mice expressing hCD59 in endothelial cells with a transgenic vector by established methods. The vector consisted of the human ICAM-II gene promoter and *hCD59* cDNA (from 5' to 3').

Northern Blot Analysis. We isolated total RNA from multiple tissues of *ThCD59*^{RBC} and *ThCD59*^{END} mice using *TRIZOL* reagent (Invitrogen) and hybridized with a ^{32}P-labeleled *hCD59* cDNA probe as described previously (Buch et al. Nat. Methods 2:419 (2005)).

Immunohistochemistry. We stained serial cuts (8 μm thick) from paraffin-embedded tissue samples with the mouse anti-hCD59 monoclonal antibody Bric 229 (International Blood Group Reference Laboratory) and FITC-conjugated horse anti-mouse secondary antibody. We used a double immunofluorescence method to investigate whether ILY passes through the blood barrier. Three hours post i.v injection with 750 ng/g of ILY or phosphate-buffered saline (PBS), we sacrificed WT mice and perfused with PBS, then fixed the tissues in formalin and subjected to double immunofluorescence labeling of tissue macrophages with anti-CD68 MAb (Serotec Inc.) and FITC-conjugated secondary antibody (Santa Crutz Biotechnology Inc.), and for recombinant ILY with anti-His MAb (Novagen) and Rhodamine-conjugated secondary antibody (Santa Crutz Biotechnology Inc.). We analyzed the co-localization of ILY and anti-CD68 MAb with filter set No. 2 for FITC and filter set No. 3 for Rhodamine using a Nikon Eclipse TE2000-E microscope.

FACS analysis. We incubated erythrocytes from ThCD59^{RBC} with Bric 229 for 30 min at room temperature, washed three times with 3% BSA/PBS buffer, and incubated for 30 min with a FITC-conjugated goat anti-mouse secondary antibody. We then washed the cells in PBS three times before analysis of fluorescence intensity by FACScan (Becton Dickinson).

Detection of circulating endothelial cells. We collected blood by venipuncture in syringes containing sodium citrate (105 mM) as an anticoagulant (blood : buffer = 9 : 1) and gently mixed with an equal volume of 0.1% BSA/PBS, added 25 μl of the antibody-coated Dynabeads® suspension per 1 ml blood and mixed thoroughly in a head-over-head mixer for 20 minutes. We washed Bead-bound cells four times with

PBS/0.1%BSA inside the magnet and flushed several times by a 100 µl pipette during each washing to eliminate nonspecific binding at 4°C. We stained the endothelial cells absorbed to beads with acridine orange and counted in a blinded fashion by two different investigators using a Nikon Eclipse 80i Upright Microscope with DIC, Phase and Epi-fluorescence Optics (Nikon Instruments Inc.) using filter set No. 3.

Other Embodiments

Various modifications and variations of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific desired embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the fields of medicine, immunology, pharmacology, endocrinology, or related fields are intended to be within the scope of the invention.

All publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent publication was specifically and individually incorporated by reference.

What is claimed is:

1. A method of treating obesity in a subject comprising administering to said subject a composition comprising substantially pure intermedilysin.

2. A method of treating a proliferative disease in a subject comprising administering to said subject a composition comprising substantially pure intermedilysin.

3. A method for treating a skin-related disorder comprising topically administering to said subject a composition comprising substantially pure intermedilysin.

4. The method of any one of claims 1, 2, and 3, wherein said substantially pure intermedilysin is administered topically.

5. The method of claim 2, wherein said proliferative disease is characterized by neoplastic cells expressing CD59.

6. The method of claim 5, wherein said proliferative disease is melanoma.

7. The method of any one of claims 1, 2, and 3, wherein said substantially pure intermedilysin is recombinant.

8. A composition formulated for contraceptive use comprising substantially pure intermedilysin.

9. A pharmaceutical composition formulated for topical administration comprising substantially pure intermedilysin.

10. The composition of any one of claims 8 or 9, wherein said substantially pure intermedilysin is recombinant.

11. A method for preventing pregnancy in a human comprising administering into the reproductive tract of said human an effective contraceptive amount of substantially pure intermedilysin.

12. The method of claim 11, wherein said substantially pure intermedilysin is administered by topical administration to the female genitalia of said human prior to sexual intercourse.

13. The method of 12, wherein said substantially pure intermedilysin is administered in combination with a pharmaceutically effective excipient, carrier, or diluent, to form a cream, lotion, gel, spray, ointment, paste, jelly or foam.

14. The method of claim 11, wherein said substantially pure intermedilysin is administered by applying said substantially pure intermedilysin on a male condom, a female condom, a contraceptive diaphragm or a contraceptive sponge, prior to sexual intercourse.

15. The method of claim 11, wherein said substantially pure intermedilysin is administered by application of said substantially pure intermedilysin on a tampon or pessary.

16. The method of claim 11, wherein said substantially pure intermedilysin is administered in the form of a contraceptive membrane suppository.

17. A method of killing a target cell comprising expressing human CD59 in said target cell, administering intermedilysin to said target cell thereby killing said target cell.

18. The method of claim 17, wherein said target cell is in a non-human animal.

19. The method of claim 18, wherein said non-human animal is a mammal selected from a group consisting of a mouse, rat, sheep, primate, dog, cat, guinea pig, cow, horse, and hamster.

20. The method of claim 18, wherein said non-human animal is a transgenic animal comprising cells comprising a human CD59 gene.

21. The method of claim 20, wherein said cells conditionally express human CD59 protein.

22. The method of claim 21, wherein said transgenic animal further comprises a Cre recombinase gene, wherein Cre recombinase protein is conditionally expressed, and wherein expression of said Cre recombinase protein results in expression of said human CD59 protein.

23. The method of claim 22, wherein said Cre recombinase gene is under the control of a tissue- or cell type-specific promoter.

24. A transgenic non-human animal comprising a gene encoding human CD59.

25. The transgenic non-human animal of claim 25, wherein human CD59 protein is conditionally expressed.

26. The transgenic non-human animal of claim 26, wherein human CD59 protein expression is tissue- or cell type-specific.

27. The transgenic non-human animal of claim 27, wherein said human CD59 protein is specifically expressed in a tissue selected from neural tissue, hematopoietic tissue, skin, endothelial tissue, and muscular tissue.

28. The transgenic non-human animal of claim 26, wherein human CD59 protein expression occurs at a specific developmental stage in a specific cell type.

29. The transgenic non-human animal of claim 26, further comprising a Cre recombinase gene, wherein Cre recombinase protein is conditionally expressed, and wherein expression of said Cre recombinase protein results in expression of said human CD59 protein.

30. The transgenic non-human animal of claim 30, wherein expression of Cre recombinase protein is under the control of a tissue specific promoter.

31. The transgenic non-human animal of claim 31, wherein said tissue specific promoter expresses said Cre recombinase protein in a tissue selected from neural tissue, hematopoietic tissue, skin, endothelial tissue, and muscle tissue.

Figure 1A.

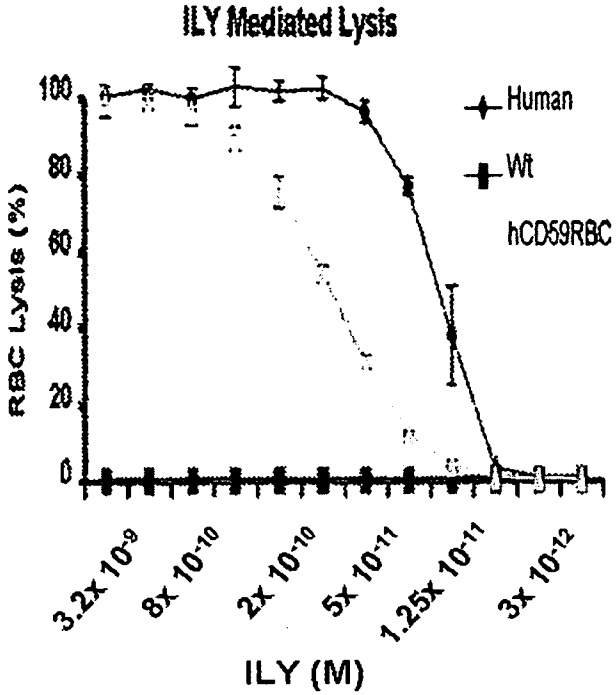


Figure 1B.

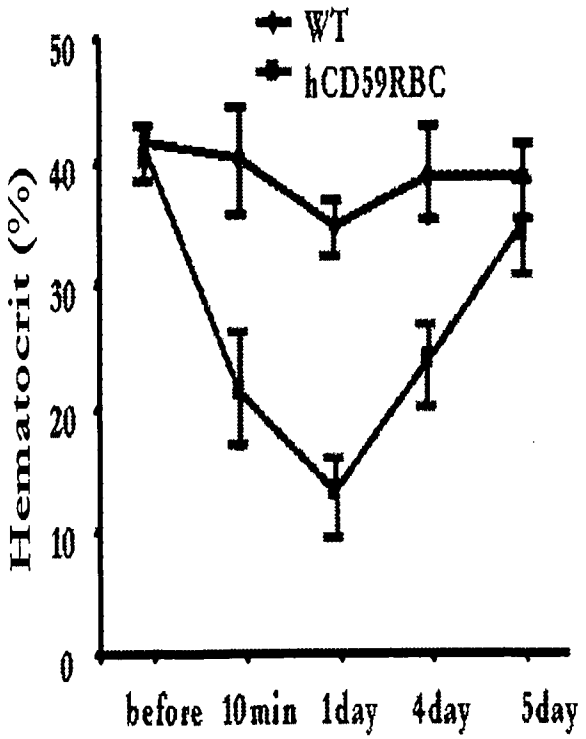


Figure 1C.

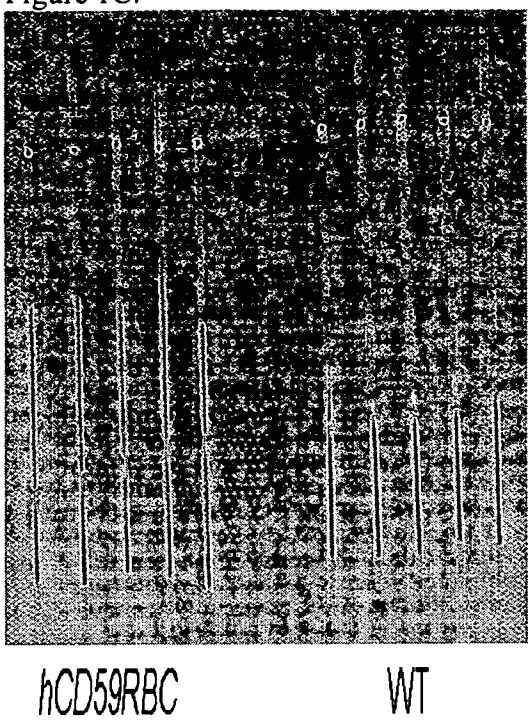


Figure 1D.

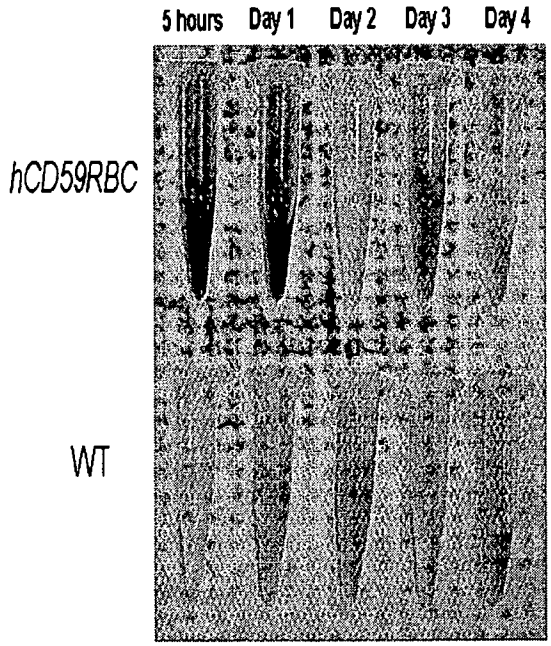


Figure 2A.

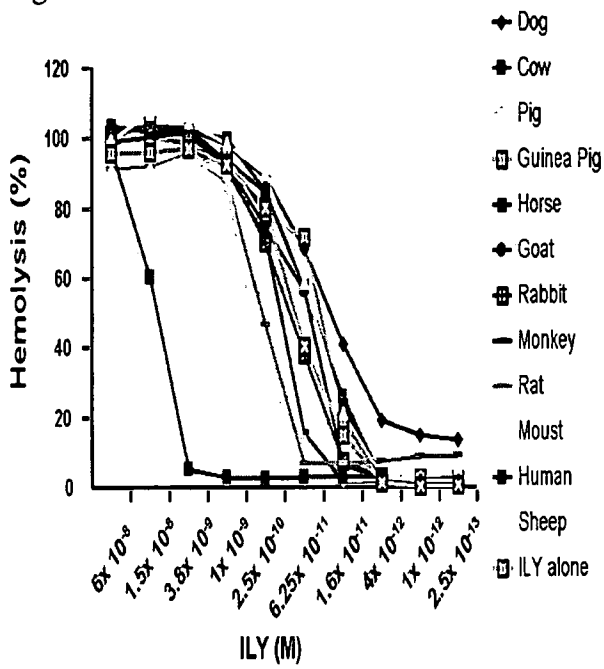


Figure 2B.

Inhibiting effect of the eluted fraction from the ILY-bound column

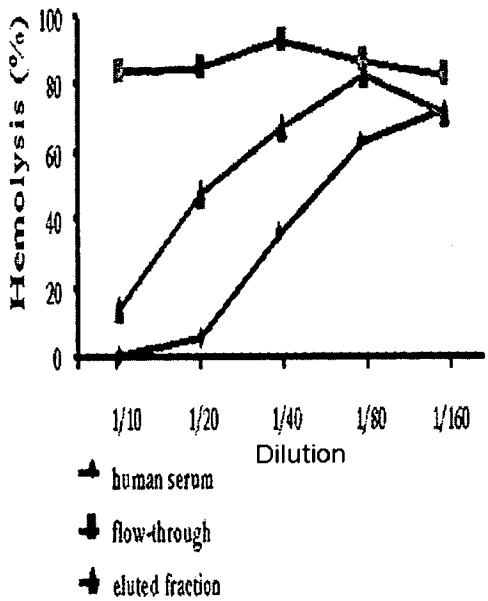


Figure 2C.

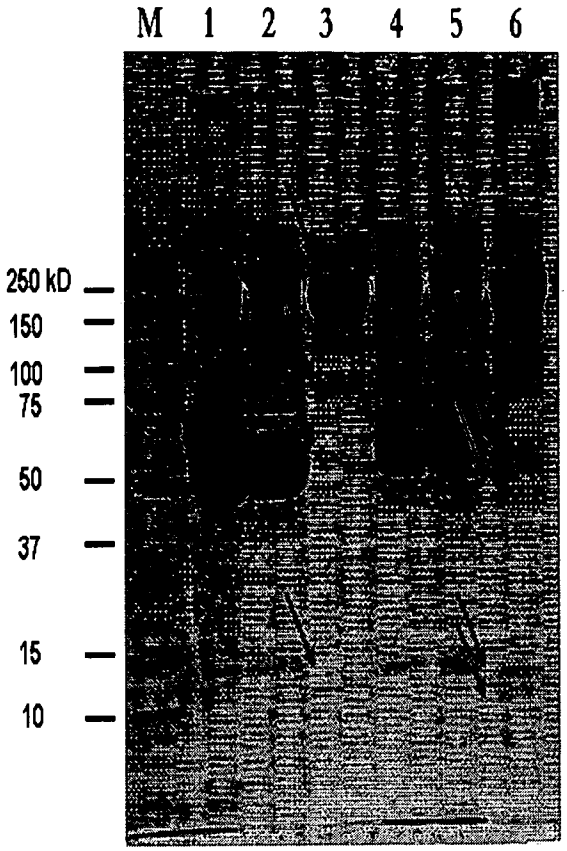


Figure 2D.

Protein sequencing information

MW(kD)	Human	Mouse
150	IgG	IgG
50/90	MAC-2 BP	

Figure 3A.

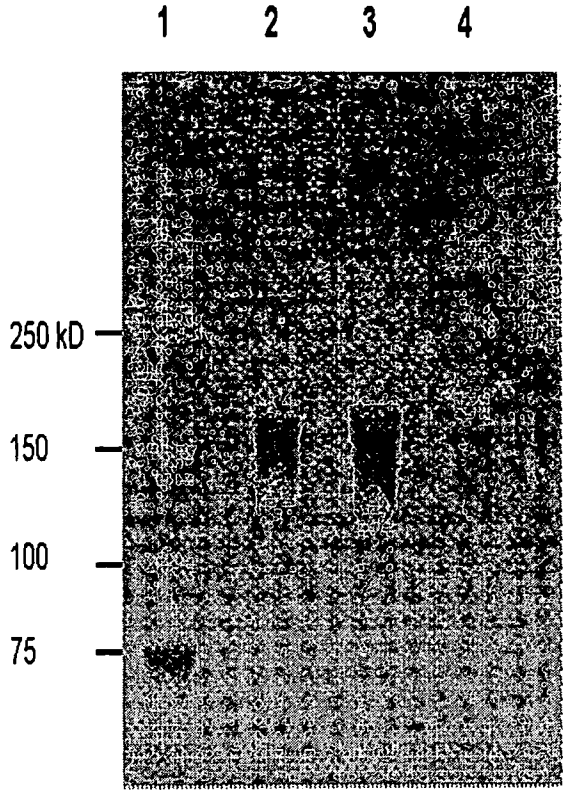


Figure 3B.

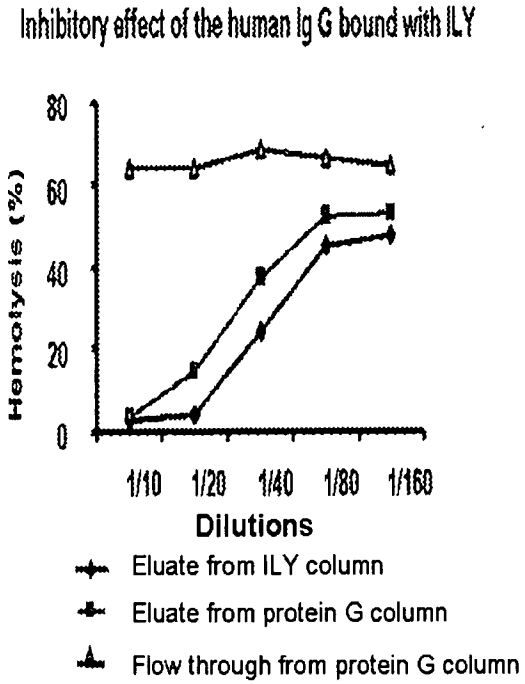


Figure 3C.

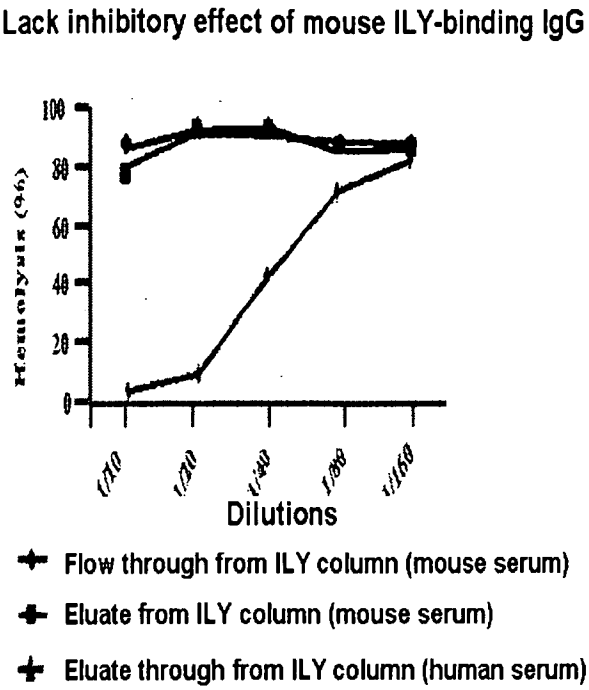


Figure 3D.

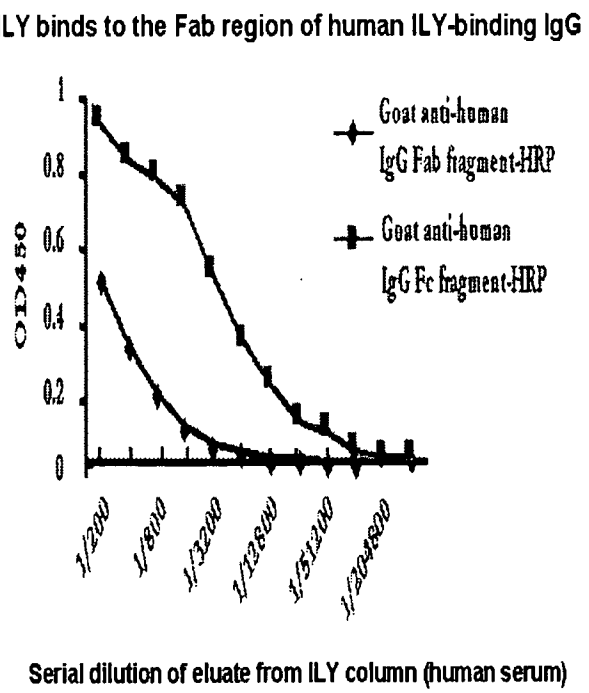


Figure 4A

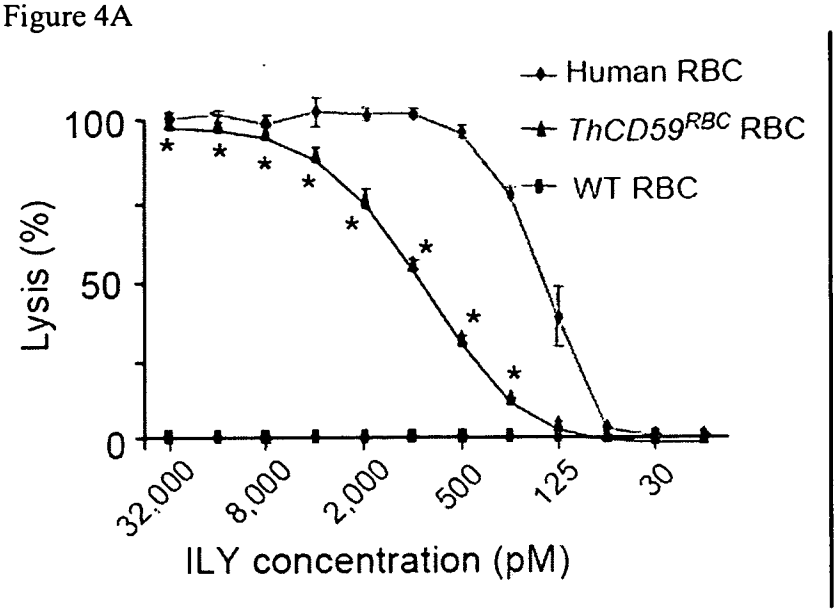


Figure 4B

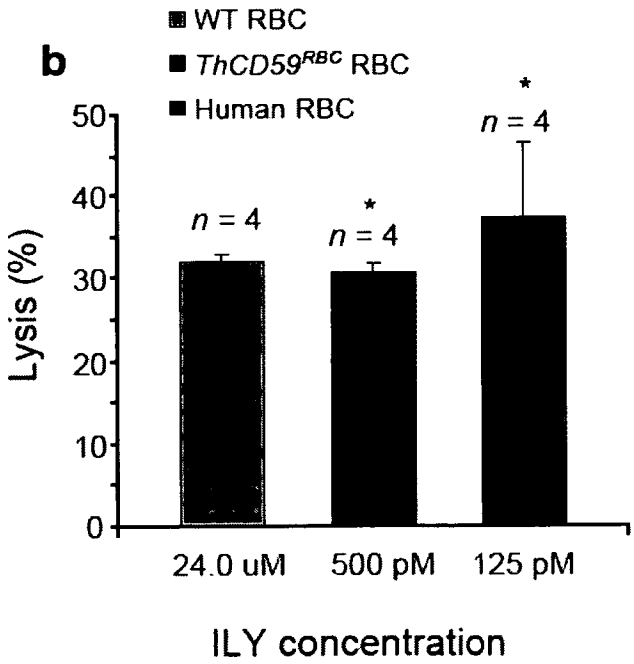


Figure 4C

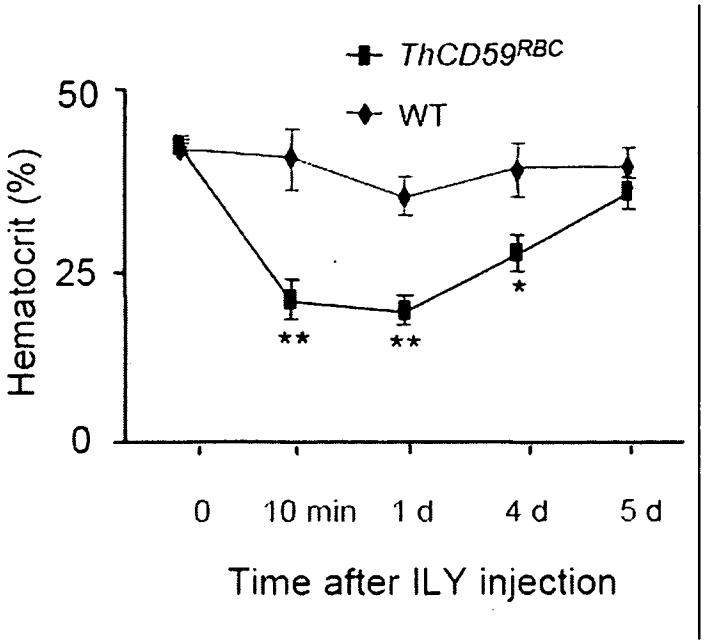


Figure 4D

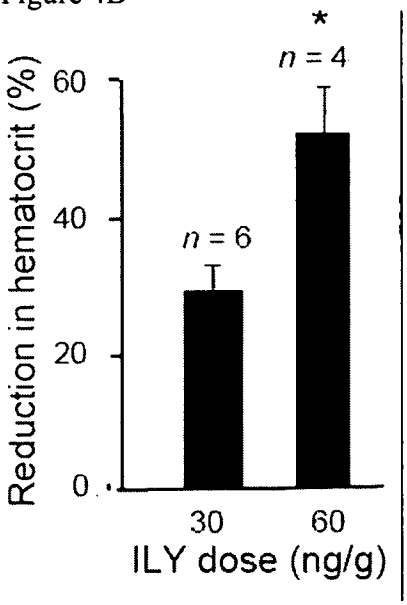


Figure 4E

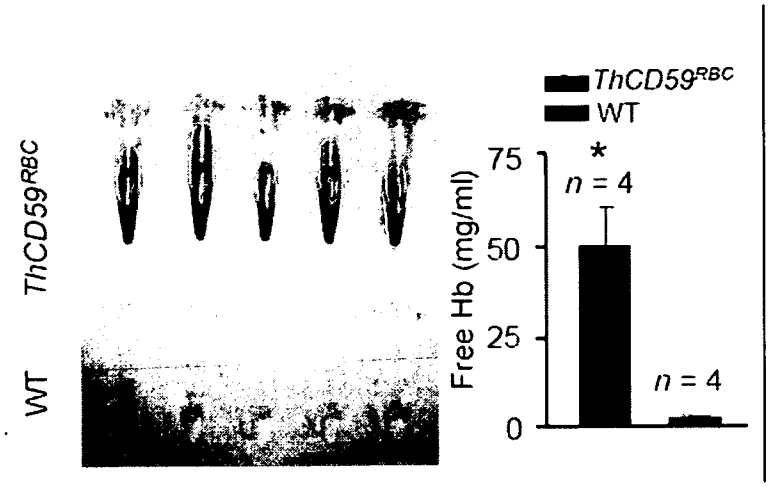


Figure 4F

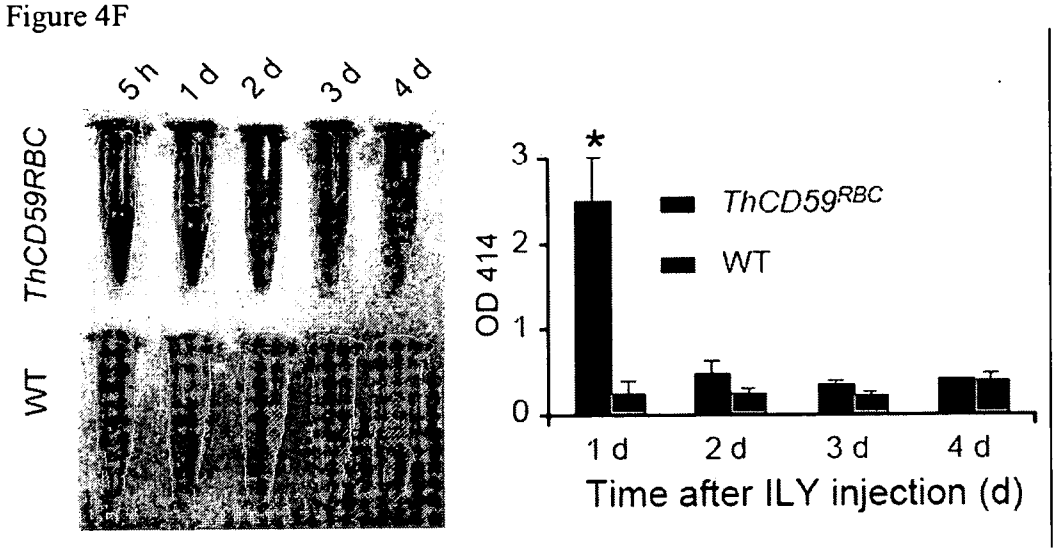


Figure 5A

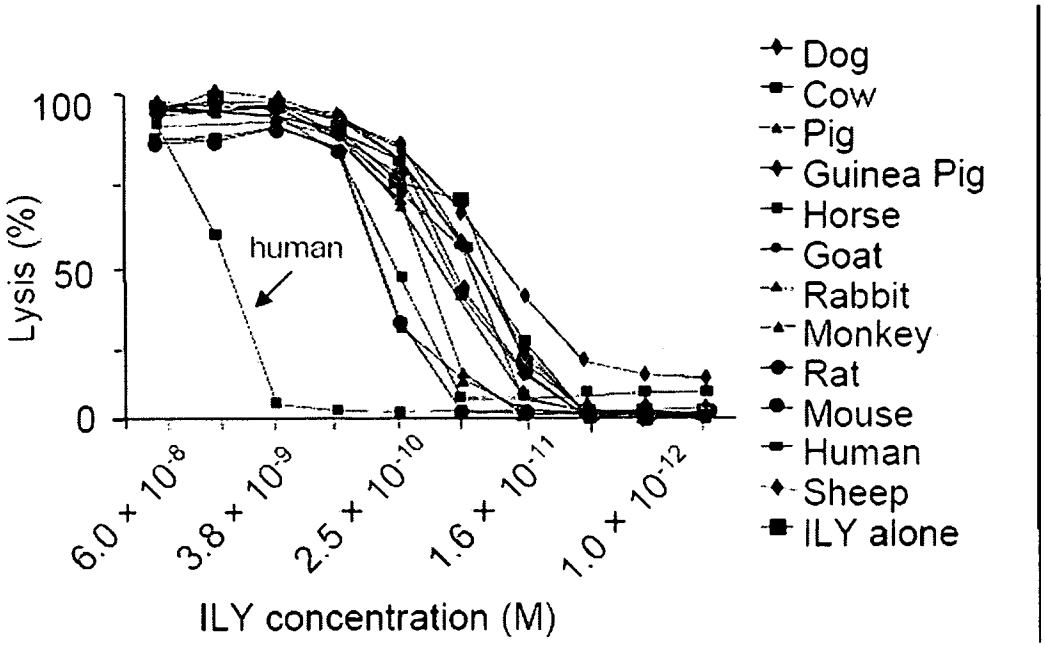


Figure 5B

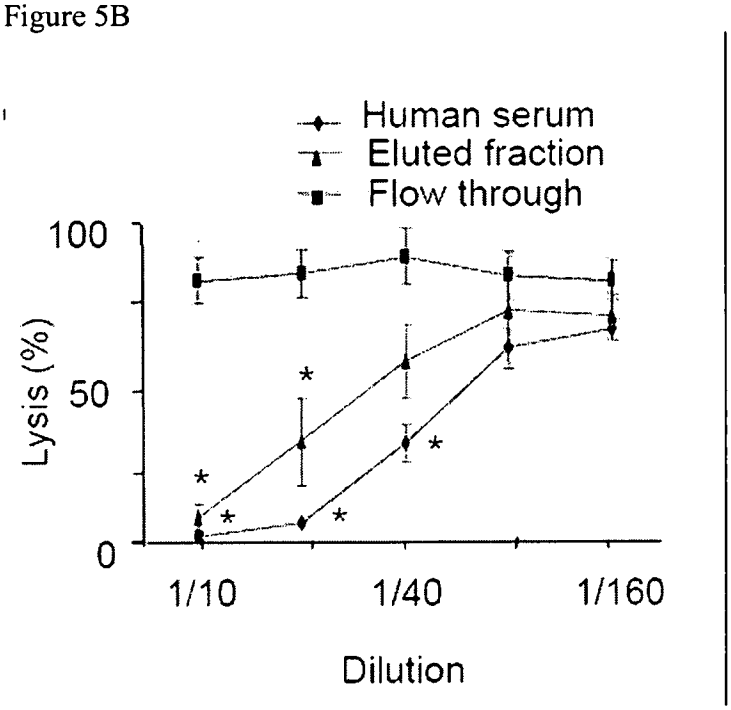


Figure 5C

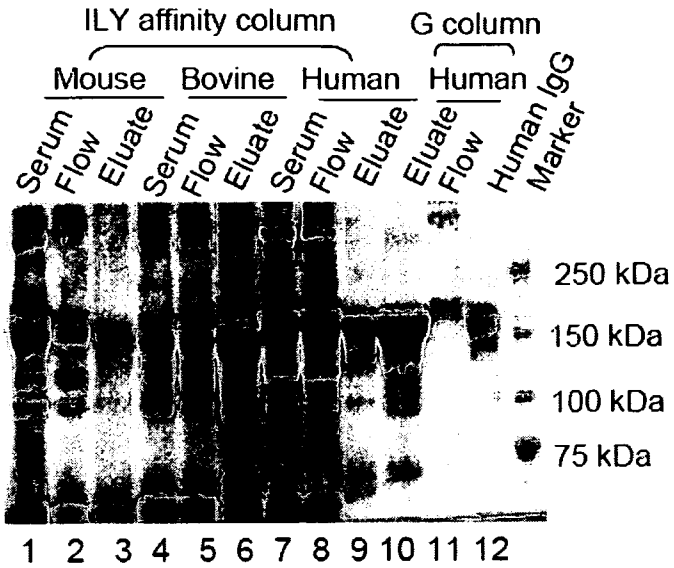


Figure 5D

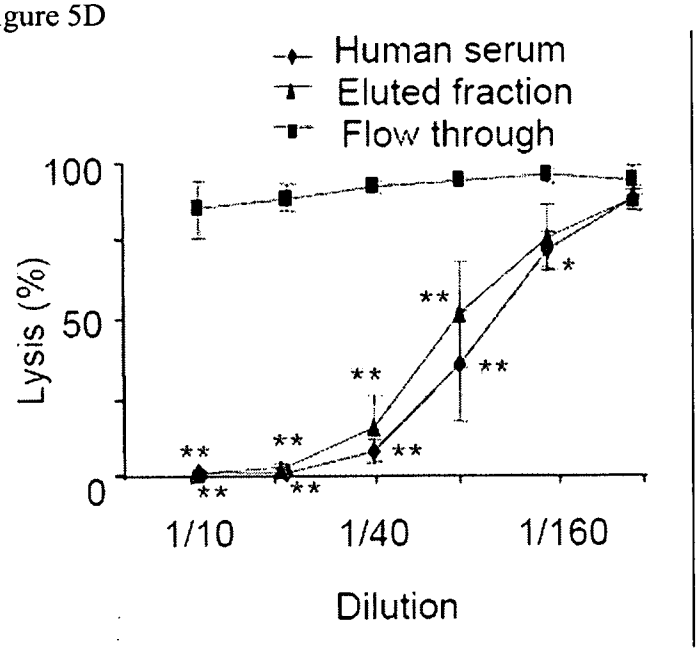


Figure 5E

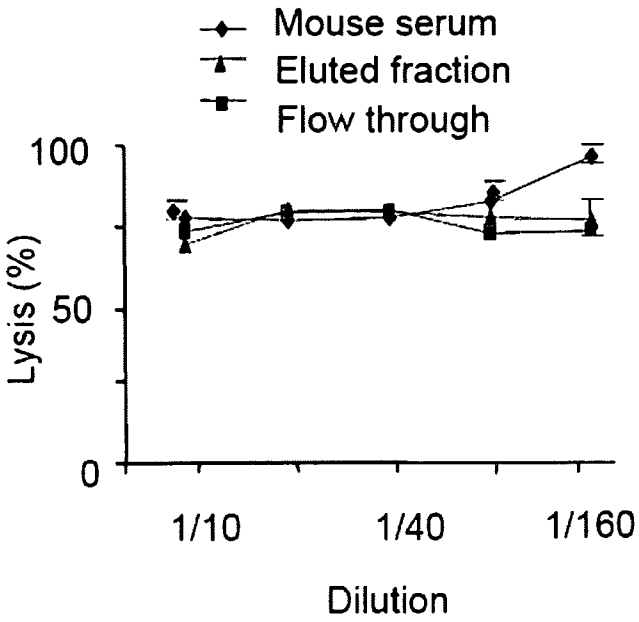

Mouse serum
Eluted fraction
Flow through
100
50
0
Lysis (%)
1/10
1/40
1/160
Dilution

Figure 6

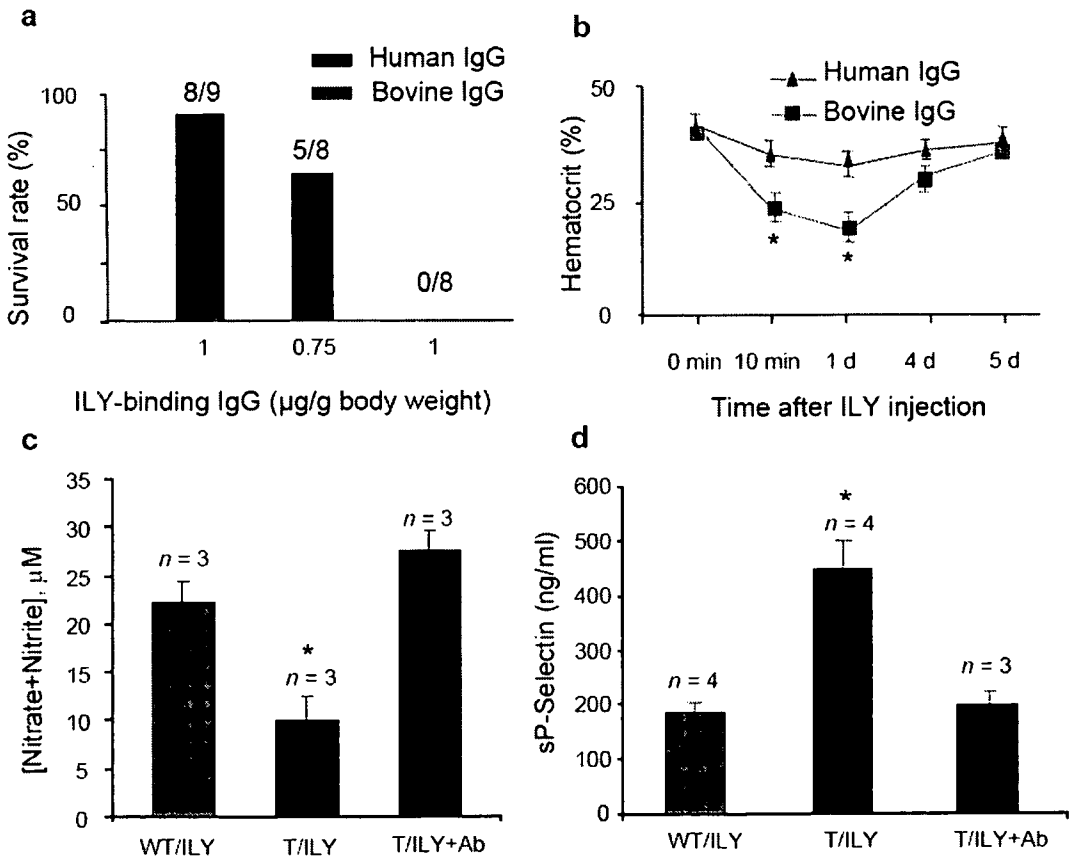

a
Human IgG
Bovine IgG
100
50
0
Survival rate (%)
8/9
5/8
0/8
1
0.75
1
ILY-binding IgG (μg/g body weight)
b
Human IgG
Bovine IgG
50
25
0
Hematocrit (%)
*
*
0 min
10 min
1 d
4 d
5 d
Time after ILY injection
c
35
30
25
20
15
10
5
0
[Nitrate+Nitrite], μM
n = 3
*
n = 3
n = 3
WT/ILY
T/ILY
T/ILY+Ab
d
600
500
400
300
200
100
0
sP-Selectin (ng/ml)
n = 4
*
n = 4
n = 3
WT/ILY
T/ILY
T/ILY+Ab

Figure 7

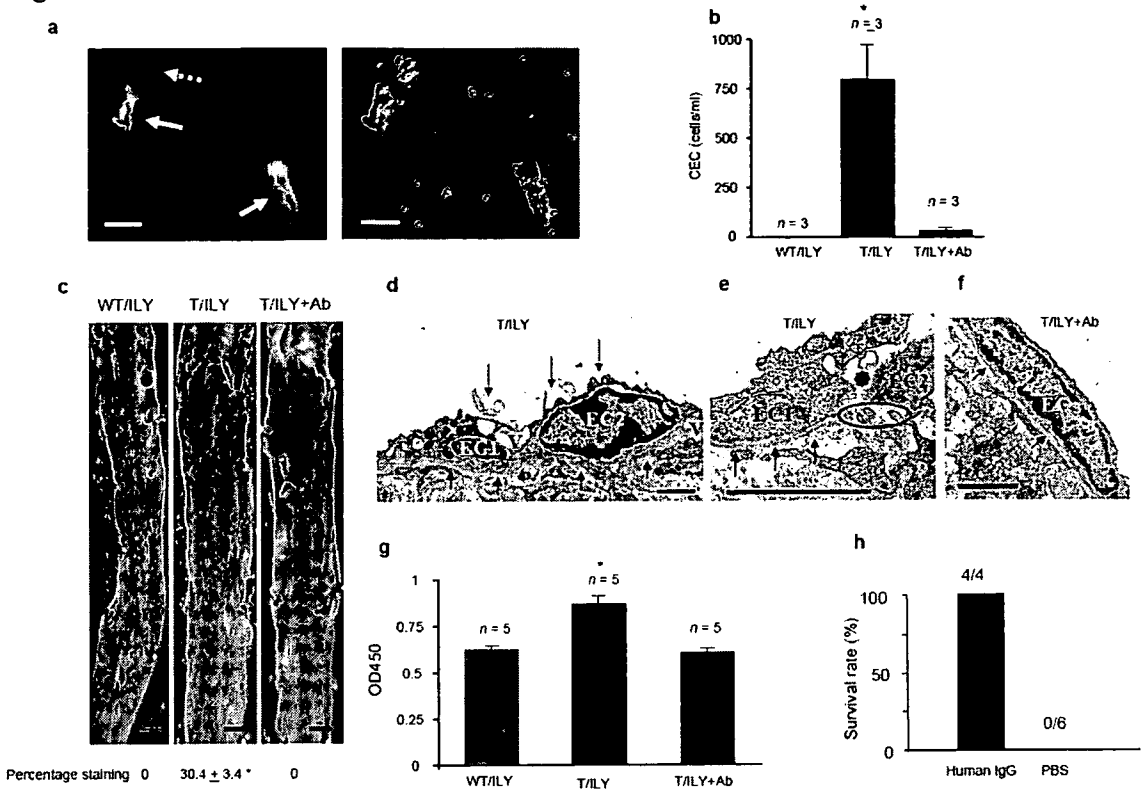

a
b
n = 3
1000
750
500
250
0
CEC (cells/ml)
n = 3
n = 3
WT/ILY
T/ILY
T/ILY+Ab
c
WT/ILY
T/ILY
T/ILY+Ab
Percentage staining 0
30.4 ± 3.4 *
0
d
T/ILY
e
T/ILY
f
T/ILY+Ab
g
1
0.75
0.5
0.25
0
OD450
n = 5
n = 5
n = 5
WT/ILY
T/ILY
T/ILY+Ab
h
4/4
100
50
0
Survival rate (%)
0/6
Human IgG
PBS

Figure 8

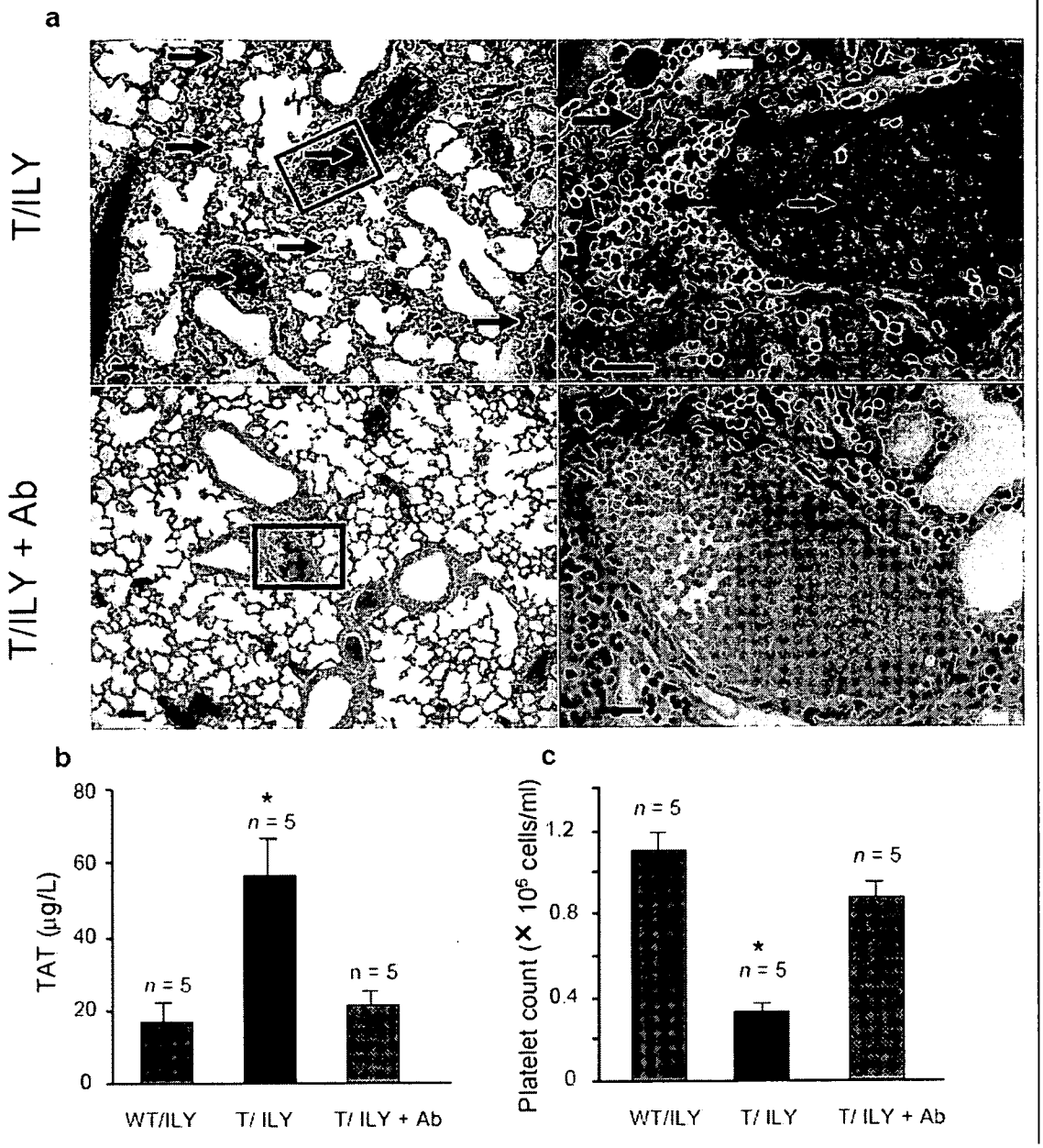

a
T/ILY
T/ILY + Ab
b
TAT (μg/L)
80
60
40
20
0
n = 5
*
n = 5
n = 5
WT/ILY
T/ ILY
T/ ILY + Ab
c
Platelet count (× 10⁶ cells/ml)
1.2
0.8
0.4
0
n = 5
*
n = 5
n = 5
WT/ILY
T/ ILY
T/ ILY + Ab

Figure 9

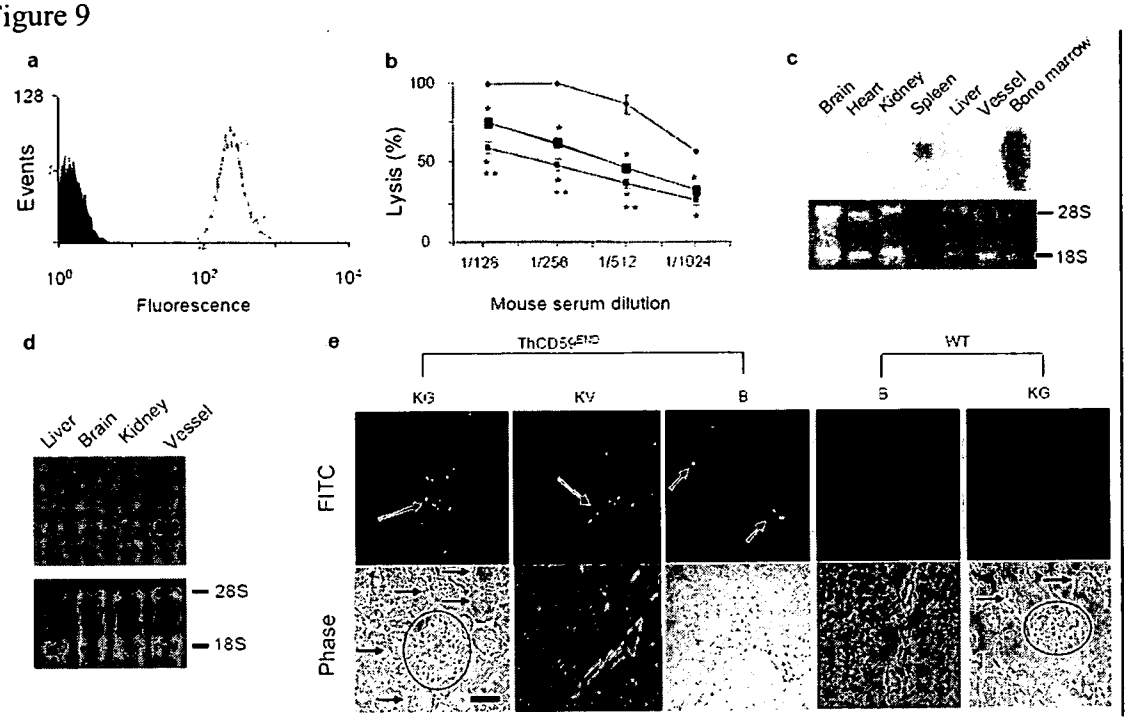


Figure 10

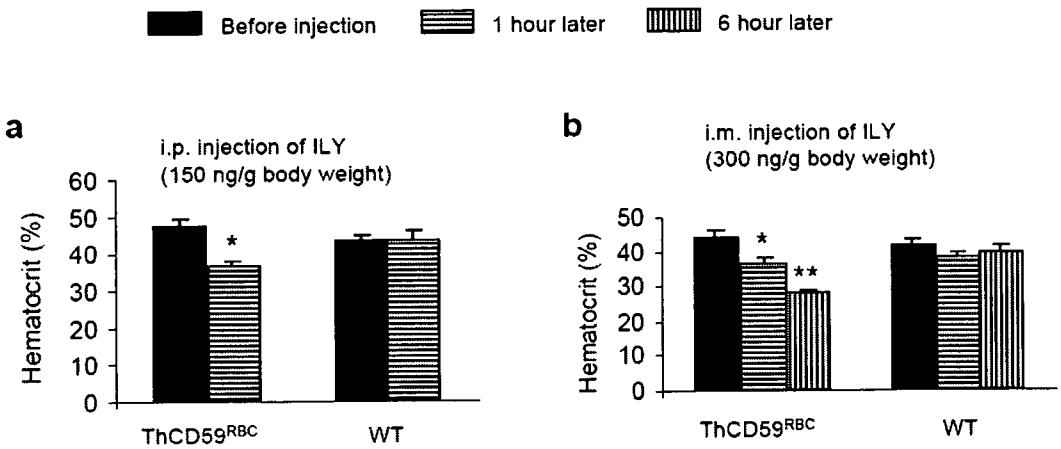
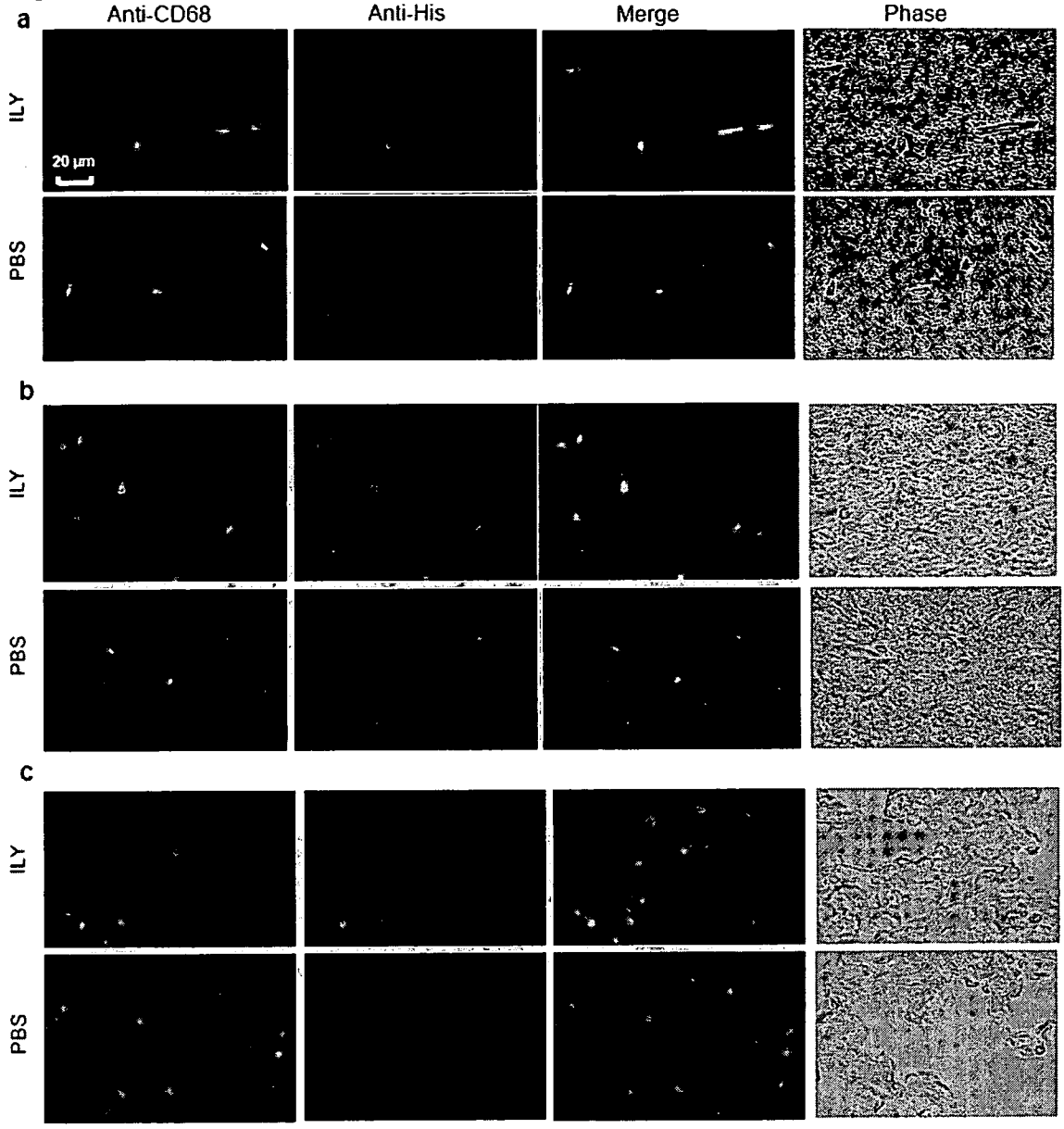


Figure 11


a
Anti-CD68
Anti-His
Merge
Phase
ILY
PBS
20 μm
b
ILY
PBS
c
ILY
PBS