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A monoclonal antibody and a method of use for the treatment of lupus

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ABSTRACT

The present invention relates to compositions and methods useful for the treatment of lupus using a humanized IgG1 anti-CD6 monoclonal antibody (T1h) that binds to the SRCR domain 1(D1) of CD6 without blocking the interaction of CD6 with the CD6 ligand Activated Leukocyte Cell Adhesion Molecule (ALCAM).

A MONOCLONAL ANTIBODY AND A METHOD OF USE FOR THE TREATMENT OF LUPUS

This is a divisional application of Australian patent application No. 2017344462, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a humanized IgG1 isotype anti-CD6 monoclonal antibody (T1h) that binds to the Scavenger receptor cysteine-rich (SRCR) domain 1(D1) of CD6 present on the surface of thymic epithelial cells, monocytes, activated T-cells and a variety of other cells types. The invention further relates to methods of inhibiting proliferation of T-cells without blocking the interaction of CD6 with the CD6 ligand Activated Leukocyte Cell Adhesion Molecule (ALCAM). It also relates to compositions and methods useful for the treatment of lupus using the anti-CD6 monoclonal antibody that binds to the SRCR domain 1(D1) of CD6.

BACKGROUND OF THE INVENTION

Lupus, a prototype of human systemic autoimmune disease, is characterized by a wide variety of multi-organ injuries. It is an autoimmune disease involving antibodies that attack connective tissue. The disease is estimated to affect nearly 1 million Americans, primarily women between the ages of 20-40. The principal form of lupus is a systemic one (systemic lupus erythematosus; SLE) and is associated with the production of antinuclear antibodies, circulating immune complexes, and activation of the complement system. While the pathogenesis of SLE is still not well understood, it is known that B cells, T-cells and monocytes are implicated in playing a critical role in the progression of the disease. Specifically, there is a marked increase in polyclonal B-cell and T-cell activity and such increase can be characterized by the development of T-cells and antibody responses against a variety of self antigens. It is theorized that the activation of T-cells stimulates the production of auto reactive B-cells to a specific epitope and then can spread to other epitopes. Such antibody response may include, as stated above, the production of autoantibodies against self antigens such as anti-nuclear antibodies (ANA) and anti-double stranded DNA antibodies.

SLE can be treated by modulating the immune response by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity or directly inhibiting the immune response are effective ways to ameliorate immune related diseases.

CD6 is an important cell surface protein predominantly expressed by human T-cells and a subset of B-cells, as well as by some B-cell chronic lymphocytic leukemias and neurons. CD6 is a member of a large family of proteins characterized by having at least one domain homologous to the scavenger receptor cysteine-rich domain (SRCR) of type I macrophages. Blocking studies using anti-CD6 monoclonal antibodies (mAbs) suggest that CD6 plays an important role in T-cell development by regulating T-cell adhesive interactions with thymic epithelial (TE) cells.

Additional studies have shown that CD6 can function as an important accessory molecule in T-cell activation. For example, certain anti-CD6 mAb are directly mitogenic for T-cells [1, 2], whereas others are able to co-stimulate T-cell proliferation in conjunction with anti-CD3, anti-CD2 or phorbol 12 myristate 13 acetate (PMA) [1, 3, 4]. Yet additional evidence of the role of CD6 in T-cell activation comes from studies showing that CD6 becomes hyperphosphorylated on Ser and Thr residues [5, 6, 7] and phosphorylated on Tyr residues [8] following T-cell activation. These and other studies implicate CD6 as an important modulator of both immature and mature T-cell function in vivo, affecting both T-cell activation and signal transduction.

The extracellular domain of the mature CD6 protein is composed of three SRCR domains (hereinafter designated D1, D2, and D3). D3 corresponding to the membrane proximal SRCR domain followed by a short 33-amino-acid stalk region. These extracellular domains are anchored to the cell membrane via a short transmembrane domain followed by a cytoplasmic domain of variable length [19].

Studies using CD6-immunoglobulin fusion proteins, containing selected extracellular domains of CD6 fused to human IgG_1 constant domains (CD6-Rgs), led to the

identification and cloning of a CD6 ligand, designated "activated leukocyte cell adhesion molecule" (ALCAM) [11, 12]. ALCAM binds to domain 3 of CD6 corresponding to the membrane proximal SRCR domain [13].

Studies of the role of CD6/ALCAM interactions in T-cell regulation have shown that this receptor-ligand pair is able to mediate the adhesion of CD6 expressing cells to thymic epithelial cells [12]. This and other evidence suggests that CD6/ALCAM interactions are important for modulating T-cell development and activation.

Although the functional characterization of CD6 remains incomplete, an anti-CD6 mAb has been successfully applied in a clinical setting to purge bone marrow of T-cells and T-cell precursors. These findings further support the hypothesis that CD6 plays an important role in modulating T-cell function in vivo. CD6 is also reported to be part of the immunologic synapse mediating early and late T-cell-antigen presenting cells (APC) interaction.[14]

U.S. Patent No. 6,372,215 discloses antibodies and other binding agents that bind specifically to SRCR domains 3 (D3) of human CD6 (hCD6) or human CD6 stalk domain (CD6S) and inhibit activated leukocyte cell adhesion molecule (ALCAM) binding to CD6.

Earlier publications and patents disclosed sequences of the murine anti-CD6 (IOR-T1) monoclonal antibody and the amino acid modifications that were carried out to humanize IOR-T1 to T1h (humanized IOR-T1). U.S. Patent No. 5,712,120 and its equivalent EP 0699755 disclose specific methods to humanize murine monoclonal antibodies and the sequence of IOR-T1 and T1h. U.S. Patent No. 6,572,857 and its equivalent EP 0807125 disclose the sequence of IOR-T1 and T1h (humanized IOR-T1). The Roque-Navarro publication [15] discusses specific methods to humanize murine monoclonal antibodies and the sequence of IOR-T1 and T1h. PCT/IN2008/00562 entitled `A Monoclonal Antibody and a Method Thereof_ discusses the targeting of CD6 as a treatment of an autoimmune disease such as multiple sclerosis, transplant rejection and graft-versus-host diseases.

There is an urgent need for improved therapeutic methods and compositions for treatment of lupus. At present, lupus is typically treated with corticosteroids and immunosuppressants. In some embodiments, antibodies are used that significantly deplete lymphocytes and in other embodiments the lymphocytes are not depleted. It would be advantageous to provide an anti-CD6 monoclonal antibody that inhibits T-cell activation by binding to CD6, the D1 domain, without interfering with the binding of ALCAM to CD6 and wherein the anti-CD6 monoclonal antibody has the ability to treat lupus and inhibit proliferation of T-cells that usually occurs in a lupus type disease.

SUMMARY OF THE INVENTION

The present invention relates to an anti-CD6 monoclonal antibody (T1h) that reduces or prevents the activation of T-cells, inhibits T-cell proliferation, reduces induction of complement-dependent cytotoxicity (CDC) and binds to domain 1 (D1) of CD6 without interfering with ALCAM binding to CD6 and wherein the anti-CD6 monoclonal antibody comprises amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2 or sequences having at least about 97% identity thereto.

In one aspect the present invention provides for a method of treating lupus, the method comprising administering to a subject suffering from the effects of lupus a therapeutically effective amount of an anti- CD6 monoclonal antibody comprising or consisting of amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

The methods of this invention can be used to treat a subject who has one or more manifestations or systems of lupus, including, without limitation, systemic lupus erythematosus, lupus nephritis, cutaneous lupus erythematosus, central nervous system (CNS) lupus, cardiovascular manifestations, pulmonary manifestations, hepatic manifestations, haematologieal manifestations, gastrointestinal manifestations, musculoskeletal manifestations, neonatal lupus erythematosus, childhood systemic lupus erythematosus, drug- induced lupus erythematosus, anti-phospholipid syndrome, or complement deficiency syndromes resulting in lupus manifestations.

In another aspect, the present invention provides for a polynucleotide encoding an anti-CD6 monoclonal antibody comprising amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2, a vector including the polynucleotides encoding the amino acid sequences, and a host cell including the vector. The cell may be eukaryotic (e.g., mammalian such a human, mouse, monkey or rabbit cell) or may be prokaryotic (e.g., a bacterial cell such as an E. coli cell).

In yet another aspect, the present invention provides for a method of treating lupus in a subject, the method comprising administering to the subject an anti-CD6 monoclonal antibody comprising amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2 or antigen-binding fragment thereof, wherein the use of the anti-CD6 monoclonal antibody shows a reduction in pro inflammatory cytokines.

Another aspect of the present invention provides for a method for modulating inflammatory conditions using an anti-CD6 monoclonal antibody comprising amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2. The monoclonal antibody may be combined with is a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial drug, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

The immunosuppressant may include prednisone, methotrexate, azathioprine or cyclophosphamide. Importantly, the administration of the anti-CD6 monoclonal antibody of the present provides for a reduced amount of immunosuppressant thereby avoiding the negative effects of immunosuppressants that can weaken the body's defense against other potential pathogens, thereby making the subject extremely susceptible to infection and other potentially fatal diseases, such as cancer.

In another aspect, the present invention also provides for a method to reduce activation of T-cells in a subject with an elevated level of anti- nuclear antibodies (ANA) and/or anti-double-stranded DNA (dsDNA) antibodies, comprising administering to the patient a therapeutically effective amount of the anti-CD6 monoclonal antibody of the present invention.

In yet a further aspect, the present invention also provides methods of treating a lupus patient in need thereof, comprising administering to the patient a therapeutically effective amount of an anti-CD6 antibody in combination with at least a second compound. The second compound is typically a therapeutic agent that is used to treat lupus, for example, a standard-of-care or experimental treatment. In the combination therapy methods of this invention, the anti-CD6 antibody and the additional therapeutic agent can be administered in any order as appropriate for the patient. The anti-CD6 antibody and the additional agent(s) can be administered concurrently or sequentially. For example, the additional agent(s) can be administered before or after the anti-CD6 therapy. Also provided in this invention are kits useful for such combination therapy.

Another aspect of the present invention provides for a monoclonal antibody which specifically binds to Scavenger receptor cysteine-rich (SRCR) domain 1(D1) of CD6 which comprises heavy chain and light chain encoded by the nucleotide sequence set forth in SEQ ID NO: 3 or a complement thereof; and (b) a nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 4 or a complement thereof.

Yet another aspect of the present invention provides for the use of the anti-CD6 antibody described above in the manufacture of medicament useful for the treatment of lupus.

Another aspect of the present invention provides for a treatment method to a subject who does not have an autoimmune disease other than lupus.

In a further aspect, the present invention provides for an article of manufacture comprising: (a) a container comprising the anti-CD6 monoclonal antibody of the present invention,; and (b) a package insert with instructions for treating lupus in a subject, wherein the instructions indicate that an amount of the antibody is administered to the subject that is effective to provide reduce the negative effects of lupus.

The foregoing and other objects and aspects of the present invention are explained in detail in the drawings herein and the specification set forth below.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the external examination of lymph nodes of normal and SLE mouse: SLE mice (right panel) shows swollen lymph nodes (marked with arrows) and enlarged salivary glands

Figure 2 shows comparison of organ weights and lymphadenopathy: Animals (n=6) were treated with 60 or 600ı g of -mCD6 or 60ı g Rat IgG (Isotype control) intraperitoneally (i.p.) for 10 Days (3 weeks per dose, alternate days). At the end of study lymphadenopathy (a) was measured (Scale 0- 3; low/no swollen lymph nodes to severe) and organ weights were measured (b-d). Significant reduction observed in lymphadenopathy score (a) and size of spleen (c) and salivary glands (d) ($p<0.05$, One way ANOVA followed by Multiple comparison test).

Figure 3 shows the results of cell proliferation assay: Single cell suspensions of lymphatic cells from each group were subjected to anti-mCD3 mediated proliferation. -mCD6 treated group showed significant ($p<0.05$; One way ANOVA followed by Multiple comparison test) hypo responsiveness to anti-CD3 mediated proliferation.

Figure 4 shows the results of the cytokine analysis: Supernatants from the proliferation assay is used to measure the cytokine release by Cytokine Bead Array (CBA) analysis. -mCD6 showed significant decrease in the release of IFN- ∴($p<0.05$; Mann-Whitney test) in the treated group (both the groups were combined for analysis purpose) compared to isotype group. TNF- was also lower in the treated group as compared to Isotype treated group but the difference not statistically significant ($p<0.09$).

Figure 5 shows the results of serum ANA and anti-ds DNA antibody analysis: Serum from the isotype and -mCD6 treated mice used to analyse (1:100 dilution) the ANA and anti-ds DNA antibodies using ELISA.

Figure 6 shows the dosing regimen for treatment of mice.

Figure 7 (A) Nucleotide sequence of VH (SEQ ID NO: 3) and Vk (SEQ ID NO: 4) of T1h derived from plasmid and genomic DNA; (B) Amino acid sequence of VH (SEQ ID NO: 1) and Vk (SEQ ID NO: 2); (C) Comparison of Vk amino acid sequence disclosed in previous publications (SEQ ID NO: 5) as compared to the sequence disclosed in this patent (SEQ ID NO: 2) to highlight the sequence differences.

Figure 8 shows ELISA reading of plate tethered with CD6-Fc in the presence of T1h and ALCAM or T1h alone.

Figure 9 shows dose dependent inhibition of T1h on lymphocytes as a bar graph. The figure represents the % of inhibition of T1h on PHA activate lymphocytes at various concentration (50 ug/ml, 25 ug/ml, 12.5 ug/ml, 6.25 ug/ml). hR3 (non specific antibody) was used at the same concentration.

Figure 10 shows the cytotoxicity fold difference between Rituxan and T1h in CDC assay using Alamar Blue.

Figure 11 shows the results of HUT 78 cells treated with T1h antibody (5 ug/ml), hR3 antibody (5 ug/ml), and rapamycin (1.2 ug/ml) or without antibody (as control) that were incubated overnight at 37é in a CO_2 incubator. Cells were then treated with Annexin V labeling solution followed by flow cytometry analysis. Annexin V FITC log on horizontal axis, PI/PE texas red on vertical axis.

Figure 12 shows the results of treating PBMCs with T1h antibody (10 ug/ml), hR3 (isotype control) or without antibody (as control) and incubated for 5 days at 37éC in a CO_2 incubator. Cells were stimulated with the Tetanus toxoid before incubation. The proliferation was measured with Alamar blue dye. No inhibition of proliferation was observed in the presence of T1h.

Figure 13 shows that Raji cells are by immunofluorescence to be true B cells and also express MHC II antigens.

Figure 14 shows PBMCs proliferative in presence of mitomycin treated Raji cells. Positive control shows that PBMCs grow in presence of PHA. sT1h inhibits T-cell proliferation (significantly by t test) as compared to no antibody or hR3 controls. Each experiment is a mean and standard deviation obtained from six different wells.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for an anti-CD6 monoclonal antibody capable of binding to domain 1(D1) of CD6 and inhibits T-cell proliferation without interfering with ALCAM binding, and wherein the anti CD6 monoclonal antibody reduces inflammatory conditions due to systemic lupus. Further it has been found that the anti-CD6 monoclonal antibody does not induce complement dependent cytotoxicity (CDC) in vitro.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook, et al. MOLECULAR CLONING: A LABORATORY MANUAL, 2nd edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., (1987)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.): PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, ed. (1987)).

Definitions

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out herein.

As used herein, "Lupus" is an autoimmune disease or disorder involving antibodies that attack connective tissue. The principal form of lupus is a systemic one, systemic lupus erythematosus (SLE), including cutaneous SLE and subacute cutaneous SLE, as well as other types of lupus (including nephritis, exfrarenal, cerebritis, pediatric, non-renal, discoid, and alopecia).

As used herein, "Anti-CD6 antibody" is generally an antibody that bind specifically to SRCR domain 1 (D1) of human CD6 (hCD6). In preferred aspects of the invention, antibodies and other immunoglobulins, including native and artificially modified antibodies and antibody fragments, are provided that bind specifically to human SRCR domain 1 of CD6 and that do not interfere with the activated leukocyte cell adhesion molecule (ALCAM) binding to CD6.

As used herein, a "subject" is a human subject. Generally, such subject is eligible for treatment for lupus. For the purposes herein, such eligible subject is one that is experiencing or has experienced one or more signs, symptoms, or other indicators of lupus or has been diagnosed with lupus, whether, for example, newly diagnosed, previously diagnosed with a new flare, or chronically steroid dependent with a new flare, or is at risk for developing lupus.

As used herein, `symptoms_ or other indicators used to diagnose lupus may include rashes on the cheeks, discoid rash, or red raised patches; photosensitivity, such as reaction to sunlight; oral ulcers, such as ulcers in the nose or mouth; arthritis, such as non-erosive arthritis involving two or more peripheral joints; renal disorder, such as excessive protein in the urine; neurologic signs, such as seizures (convulsions); and hematologic symptoms, such as hemolytic anemia, leukopenia, lymphopenia or thrombocytopenia.

As used herein, `monoclonal antibody_ (mAb) refers to an antibody of a population of substantially homogeneous antibodies; that is, the individual antibodies in that population are identical except for naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single

antigenic determinant, an `epitope._ Therefore, the modifier `monoclonal_ is indicative of a substantially homogeneous population of antibodies directed to the identical epitope and is not to be construed as requiring production of the antibody by any particular method. It should be understood that monoclonal antibodies can be made by any technique or methodology known in the art; including e.g., recombinant DNA methods known in the art, or methods of isolation of monoclonal recombinantly produced using phage antibody libraries.

As used herein, "Complement-dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system to a molecule (e.g. an antibody) complexed with a cognate antigen.

As used herein, "cytokine" is a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines; interleukins (ILs) such as IL-1, IL-l , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-15; a tumor necrosis factor such as TNF- or TNF-ϕ.

As used herein, "growth-inhibitory" antibodies are those that prevent or reduce proliferation of a cell expressing an antigen to which the antibody binds. For example, the antibody may prevent or reduce proliferation of T-cells in vitro and/or in vivo.

As used herein, "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

As used herein, an "autoimmune disease" is a disease or disorder arising from and directed against an individual's own tissues or organs and results from, or is aggravated by, the production by B-cells of antibodies that are reactive with normal body tissues and antigens, such as, secretion of an autoantibody that is specific for an epitope from a self-antigen (e.g. a nuclear antigen).

As used herein, determination of apoptosis (programmed cell death) by specific antibodies, that being, those that "induce or do not induce apoptosis, e.g. of a T-cell, can be determined by standard apoptosis assays, such as binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies).

It is understood that aspects of the present invention described herein also include "consisting of" and "consisting essentially of" aspects.

According to the first aspect of the present invention, there is provided an anti-CD6 monoclonal antibody that is capable of specifically binding to D1 domain of CD6 without interfering with the binding of ALCAM to CD6 comprising SEQ ID NO: 1 and SEQ ID NO: 2. The nucleotide sequences encoding the anti-CD6 monoclonal antibody includes SEQ ID NO: 3 and SEQ ID NO: 4, respectively or nucleotide sequences have at least 90% identity thereto and encode for SEQ ID NO: 1 and SEQ ID NO: 2.

Methods for producing the anti-CD6 monoclonal antibodies of the invention

The present invention further provides methods for producing the disclosed anti-CD6 antibodies. These methods encompass culturing a host cell containing isolated nucleic acid(s) encoding the antibodies of the invention. As will be appreciated by those in the art, this can be done in a variety of ways, depending on the nature of the antibody.

In general, nucleic acids are provided that encode the antibodies of the invention. The polynucleotides can be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs, and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded, may be the coding (sense) strand or non- coding (anti-sense) strand. The coding sequence that encodes the an anti-CD6 monoclonal antibody may be identical to the coding sequence provided herein or may be a different coding sequence, which sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptides as the DNA provided herein.

In some embodiments, nucleic acid(s) encoding the anti-CD6 monoclonal antibody of the present invention are incorporated into expression vectors, which can be extrachromosomal or designed to integrate into the genome of the host cell into which it is introduced. Expression vectors can contain any number of appropriate regulatory sequences (including, but not limited to, transcriptional and translational control sequences, promoters, ribosomal binding sites, enhancers, origins of replication, etc.) or other components (selection genes, etc.), all of which are operably linked as is well known in the art. In some cases two nucleic acids are used and each put into a different expression vector (e.g. heavy chain in a first expression vector, light chain in a second expression vector), or alternatively they can be put in the same expression vector. It will be appreciated by those skilled in the art that the design of the expression vector(s), including the selection of regulatory sequences may depend on such factors as the choice of the host cell, the level of expression of protein desired, etc.

In general, the nucleic acids and/or expression can be introduced into a suitable host cell to create a recombinant host cell using any method appropriate to the host cell selected (e.g., transformation, transfection, electroporation, infection), such that the nucleic acid molecule(s) are operably linked to one or more expression control elements (e.g., in a vector, in a construct created by processes in the cell, integrated into the host cell genome). The resulting recombinant host cell can be maintained under conditions suitable for expression (e.g. in the presence of an inducer, in a suitable non-human animal, in suitable culture media supplemented with appropriate salts, growth factors, antibiotics, nutritional supplements, etc.), whereby the encoded polypeptide(s) are produced. In some cases, the heavy chains are produced in one cell and the light chain in another.

The expression vectors can be transfected into host cells such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Yeast, insect, and plant cells can also be used to express recombinant antibodies. In some embodiments, the antibodies can be produced in transgenic animals such as cows or chickens.

General methods for antibody molecular biology, expression, purification, and screening are described, for example, in Antibody Engineering, edited by Kontermann & Dubel, Springer, Heidelberg, 2001 and 2010.

Mode of Administration

For administration in the methods of use described below, the anti-CD6 monoclonal antibody may be mixed, prior to administration, with a non-toxic, pharmaceutically acceptable carrier substance (e.g. normal saline or phosphate-buffered saline), and will be administered using any medically appropriate procedure, e.g., parenteral administration (e.g., injection) such as by intravenous or intra-arterial injection.

Formulations of the anti-CD6 monoclonal antibody used in accordance with the present invention may be prepared by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers in either the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3- pentanol and m-cresol; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEENù , PLURONICSù or polyethylene glycol (PEG).

The anti-CD6 monoclonal antibody may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are well known in the art.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the anti-CD6 monoclonal antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels, copolymers of L-glutamic acid, non-degradable ethylene-vinyl acetate and degradable lactic acid-glycolic acid copolymers.

The anti-CD6 monoclonal antibody may be administered to a subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal or oral routes. Intravenous or subcutaneous administration of the anti-CD6 monoclonal antibody is preferred.

Treatment of lupus related diseases according to the present invention includes a "therapeutically effective amount" of the anti-CD6 monoclonal antibody used. Notably, a therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the anti-CD6 monoclonal antibody to elicit a desired response in the individual.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. The efficient dosages and the dosage regimens for the anti-CD6 monoclonal antibodies used in the

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present invention depend on the severity of the lupus-type disease and may be determined by the persons skilled in the art.

An exemplary, non-limiting range for a therapeutically effective amount of the anti-CD6 monoclonal antibody used in the present invention is about 0.01-100 mg/kg per subject body weight, such as about 0.01-50 mg/kg, for example about 0.01-25 mg/kg. A medical professional having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, a physician could start doses of the anti-CD6 monoclonal antibody at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In one embodiment, the anti-CD6 monoclonal antibody is administered by infusion in a weekly dosage of from 1 to 500 mg/kg per subject body weight, such as, from 20 to 200 mg/kg. Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. In the alternative, the administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as, from 2 to 12 hours.

In one embodiment the anti-CD6 monoclonal antibody is administered in a weekly dosage of from 10 mg to 200 mg, for up to 7 times, such as from 4 to 6 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as, from 2 to 12 hours. Such regimen may be repeated one or more times as necessary, for example, after 6 months or 12 months.

The Examples which follow are set forth to aid in understanding the invention but are not intended to, and should not be construed to limit its scope in any way. The Examples do not include detailed descriptions for conventional methods employed in the assay procedures. Such methods are well known to those of ordinary skill in the art and are described in numerous publications including by way of examples.

Example 1

CD6 is a co-stimulatory molecule, predominantly expressed on lymphocytes and is associated with many autoimmune disease [16, 17]. The anti-mouse CD6 (-mCD6) binds specifically to domain 1 of CD6 and is a surrogate antibody for Itolizumab. Previous studies using this antibody demonstrated significant amelioration of EAE (Experimental Autoimmune Encephalomyelitis) a model for multiple sclerosis in mice. In the present study, the antibody is evaluated in MRL $^{Fas/lpr}$ mice, a relevant mouse model for lupus like disease in humans. -mCD6 treatment showed significant reduction in lymphadenopathy, spleen and salivary glands weights ($p<0.05$) compared to isotype control. -mCD6 treated animals showed significant hypo proliferation to anti-CD3 mediated T-cell proliferation assays with concurrent reduction in release of pro inflammatory cytokines like IFN- .:(p<0.04)and TNF- (p<0.09).

MRL $^{Fas/lpr}$ is a well-known animal model used in Lupus like autoimmune diseases [18-21]. This model has spontaneous mutation in Fas gene, predominantly affecting the proliferation of B and T-cells. This mutation prevents apoptosis with uncontrolled proliferation of lymphocytes thereby resulting in massive enlargement of lymph nodes (lymphadenopathy), salivary glands and spleen as shown in Figure 1. This model also shows glomerulonephritis which resembles Lupus Nephritis in humans [22]. In mice, the disease onset is 8 week onwards till mortality between weeks 16-18 [20, 21].

Systemic Lupus Erythematosus (SLE) is an autoimmune inflammatory disease that affects mostly middle-aged women (ratio 9:1 women to men). Characteristics of SLE include skin eruptions, joint pain, recurrent pleurisy, and kidney disease.

This study describes the use of -mCD6 in this lupus model. Animals (n=6 per group, age: 12 weeks) were treated with 60 and 600 ı g/dose of -mCD6 or 60 ı g of Rat IgG (Isotype control) intraperitoneally (i.p.) for 10 Days (3 weeks per dose). Animals were sacrificed at the age of 16 weeks. The testing regime is shown in Figure 6. At the end of study the following parameters were evaluated:

- ¿ Measurement of lymphadenopathy followed by organ collection and evaluation at the end of the study.

- ¿ Proteinuria- Measured during the duration of the study.
- ¿ Blood collection at study terminus.
- ¿ Proliferation assays with lymph nodes and spleen.
- ¿ Anti-nuclear antibody and anti-ds DNA antibody measurement from mouse serum.
- ¿ Cytokine analysis from the proliferation assay.

Results:

Decrease in lymphadenopathy with associated reduction in certain lymphoid organs with -mCD6.

Lupus mice treated with -mCD6 antibody showed significant reduction in lymphadenopathy score and weight of the organs i.e., spleen, salivary glands compared to isotype control as shown in Figure 2. There was no difference in the proteinuria, kidney and thymus size measured between groups (data not shown).

-mCD6 treated shows hypo responsiveness to anti-CD3 mediated proliferation with associated reduction in pro inflammatory cytokines

Lymph node derived cells from animals treated with -mCD6 show hypo responsiveness to anti-CD3 mediated proliferation of T-cells ($p<0.05$) as compared to Isotype treated group, indicating suppression of T-cell activation probably associated with the disease suppression, as shown in Figure 3.

Cytokines play a major role in pathogenesis of SLE [23-26]. Th1/Th2/Th17 cytokines were measured from the supernatants from the proliferation assay. -mCD6 treated groups showed lower release of pro inflammatory cytokines like IFN-∴and TNF- as compared to control group, as shown in Figure 4. However, there was no difference in the other cytokines (IL-2, IL-6, IL-10 and IL-17) evaluated between the groups.

-mCD6 treated animals possibly impacts B cell response

Anti-Nuclear Antibody (ANA) and anti-ds DNA are the key auto antibodies generally observed in Lupus disease. This is also observed in this animal model [18, 20, 21, 27]. Treatment with -mCD6 showed lesser ANA and anti-ds DNA antibodies in the mice serum, as shown in Figure 5, as compared to isotype treated group. The reduction was not however statistically significant.

In this initial dose finding study both 60 and 600ı g/dose showed comparable efficacy in the physical and biological endpoints measured. This would suggest a dose saturation by 60ı g/dose. Thus, it has been shown that use of the -mCD6 antibody is able to alleviate Lupus like symptoms in this mice model.

Example 2

T1h and ALCAM does not Bind to the Same Domain on CD6 by ELISA

When varying concentrations of ALCAM-Fc was incubated along with a fixed concentration of T1h in a CD6-Fc coated ELISA plate, T1h was detected at all concentration of ALCAM-Fc. This experiment suggested that T1h binds to a different domain from the ALCAM binding domain (Domain 3).

The rhCD6FC/Chimera (R and D systems) (100 ≈g/ml) was diluted in coating buffer and 100 ≈l was added to each well of a 96 well Nunc-Maxisorp plate. The plate was then incubated at 4éC overnight. The Plate was washed thrice with PBS Tween 20. Subsequently, 200 ≈l of blocking solution (2% BSA+0.1%Tween 20 in 1x PBS) was added and incubated for 1 hour at 37éC. After incubation, the plate was washed again with PBS Tween thrice, followed by the addition of T1h monoclonal antibody (0.2 mg/ml) and .rhALCAMFc (R and D systems) at varying concentrations. This was then incubated for an hour at 37éC. The plate was washed 3 times subsequently with PBS Tween. To the wells 200 ≈l of anti human IgG $(Fab)_2$ ALP (1:20000) diluted in blocking buffer was added and incubated for 1 hour at 37éC. The plate was washed thrice with

PBS tween and 200 ≈l of p-Nitrophenyl Phosphate (PNPP) substrate is added to each well and incubated at 37éC till color develops around 15 minutes. Reading was taken at 405 nm using a BIOTEK Micro Plate Reader. The experiment indicates that the presence of ALCAM in varying concentrations does not prevent T1h from binding to a CD6 receptor. The absence of competition between ALCAM and T1h suggests that the binding domains for the two are different, as shown in Figure 8.

Example 3

Lymphocyte Proliferation Inhibition by Flow Cytometry Using CFSE

PBMCs were harvested and washed in PBS. The cells (7.5 x 10^6) were re-suspended in 1 ml of 2 ≈M carboxyfluorescein succinimidyl ester (CFSE) concentration in PBS. Cells were incubated for 10 minutes exactly at 37éC. 10 ml of Roswell Park Memorial Institute medium (RPMI), 10% FBS was added to stop the reaction. Cells were washed twice with 10 ml of PBS. The cell preparation was then re-suspended in 5 ml of PBS at a cell density of 1.5 x 10^6 cells/ml and 200 ul was added to each BD FACS tube. 200 ul of required and non specific antibody at various concentrations (50 ug/ml, 25 ug/ml, 12.5 ug/ml and 6.25 ug/ml respectively) were added and incubated for 30 minutes at 37éC. 2 ml of PBS was added to each tube and centrifuged at 1200 RPM for 5 minutes at RT to wash away the unbound antibody. 1 ml of RPMI, 10% FBS was added to the pellet in each tube. 1 ml of PHA 20 ≈g/ml in RPMI 10% FBS was added to the respective tube to stimulate the proliferation. The total volume in the tube is 2 ml and the final concentration of PHA was 10 ≈g/ml. The tube was vortexed and incubated for 3 day at 37éC in CO_2 incubator. Cells were washed with PBS and spun down at 1200 RPM at 4éC for 5 minutes. Supernatant were discarded and resuspended in 500 ul of 1x PBS. Total 20000 events were acquired at around 200 events/sec and viewed in the FITC channel.

% of Inhibition={[PHA-(T1h+PHA)]/PHA}*100

(Where PHA=PHA-cells alone PHA+T1h=(PHA+T1h)-Cells alone

PHA+hR3=(PHA+hR3)-Cells alone)

Cells alone is CFSE+cells.

These data suggest that T1h antibody mediates the inhibition of proliferation of PHA stimulated lymphocytes in a dose dependent manner. The percentage of inhibition may be varied among the individuals due to inherent variation among normal individuals. However overall, a dose dependent inhibition of PHA stimulated lymphocytes was observed with T1h but not with a nonspecific antibody hR3, as shown in Figure 9.

Example 4

T1h does not Mediate Complement Dependent Cytotoxicity (CDC)

The Alamar Blue (Resazurin) based assay is used to measure the ability of an antibody to promote cell killing. This is induced by the binding of the antibody to a cell surface antigen thereby fixing and activating complement resulting in target cell lysis. Resazurin is a redox-active dye which when reduced, changes color from blue to pink.

Pooled human serum (minimum three) from whole blood was collected in sterile tube the blood was allowed to clot at room temperature for at least 4 hours and is centrifuged at 900 g for 20 minutes. The serum was harvested; aliquoted and stored at -80éC. Target cells (Wil-2S/HUT-78) were washed in dilution buffer and resuspended to 2 x 10^5 cells/mL. Antibody was diluted in dilution buffer at 4 x of the final desired concentration. Complement was diluted at 4x the desired final concentration (i.e. 1:2.5 dilutions for a final concentration of 1:10). 50 ≈L each of diluted antibody, diluted complement and 50 ≈L of cell suspension (10,000 cells/well) were added to each well of a 96-well flat-bottom plate. The following control wells were included: target cells+Ab alone (spontaneous cell death), target cells+serum only (background lysis), and targets cells+10% SDS (for maximum cell death). The positive control was Wil-2S cells treated with Rituxan at different concentrations. 96-well plate was incubated for 2 hours at 37éC. 50 uL/well of Alamar Blue was added to each well, and the plate was incubated overnight

at 37éC. Fluorescence was measured on a spectrophotometer Biotek Synergy.TM. HT with 530 nm excitation, 590 nm emission, and sensitivity=35. The results suggest that T1h does not induce CDC as compared to Rituxan, as shown in Figure 10. Thus, the results from this experiment conclusively proves that the anti-CD6 monoclonal antibody does not induce CDC in a cell line expressing CD6 namely HUT 78.

Example 5

T1h does not Induce Apoptosis in HUT 78 Cells

One of the hallmarks of apoptosis is the translocation of phosphotidylserine (PS) from the inner part of the plasma membrane to the outside. The analysis of phosphotidyl serine on the outer leaflet of apoptotic cell membranes is performed by using Annexin-V-Fluorescein and Propidium iodide (PI) for the differentiation of apoptotic and necrotic cells. Annexin V is a Ca^{2+} dependent phospholipid binding protein with a high affinity for phosphotidyl serine. While PI binds to distinct necrotic cells, Annexin-V-Fluorescein binds to apoptotic cells. This method helps in distinguishing the apoptotic and necrotic cell populations. The early apoptotic population is only Annexin V positive while the late apoptosis is both Annexin V and PI positive.

The cells were harvested and 1.5 ml of 3.3 x 10^5 cells/ml (final cells: 5 x 10^5 cells) was seeded in each 35 mm dish. Required amount of antibody was added to respective dishes to make a final concentration of (5 ≈g/ml). In the control dish, no antibody was added. As a positive control cells were incubated with rapamycin at a concentration of 1.2 ≈g/ml. Cells were incubated overnight at 37éC in 5% CO_2 incubator. The cells were then transferred to FACS tube BD Falcon Cat No: 352054 and centrifuged at 1200 RPM for 5 minutes at Room temperature (RT). The supernatant was discarded and resuspended in 2 ml of PBS and centrifuged at 1200 RPM for 5 mints at RT. The supernatant was discarded and 100 ≈l of Annexin-V-Fluorescein labeling solution was added and incubated for 10-15 min at RT. The cells were washed with 2 ml of PBS and centrifuged at 1200 RPM for 5 minutes. The supernatant was then discarded. Cells were resuspended in 0.5 ml of PBS and acquired by flow cytometer (3000 cells were gated)

with 488 nm excitation. Samples were read in FITC channel for Annexin V and PE Texas red channel for PI. Annexin V alone and PI alone samples in the rapamycin treated arm, were run to enable compensation.

The HUT 78 cells that were treated with the T1h showed 40% of apoptosis which is almost equal to the untreated control in the Annexin V FITC channel. The untreated and the nonspecific antibody (hR3 antibody) treated cells showed 35.3% and 36.5% apoptosis respectively while the positive control rapamycin showed 54.3% apoptosis. This data suggest that the T1h does not mediate apoptosis in the HUT 78 cells, as shown in Figure 11.

Example 6

No Inhibition of Memory T-cells by T1h in a Tetanus Toxoid Mediated T-cell Proliferation Assay

PBMCs were isolated by Ficoll-Paque (Amersham Cat No: 17-14403-03), density gradient centrifugation. Buffy coats were obtained from healthy donors and always harvested fresh. PBMCs were then washed in PBS (Invitrogen). The PBMCs were then re-suspended in 2 ml of RPMI media with 5% FBS supplemented at a cell density of 0.3 x 10^6 cells/ml. The cells were then incubated for 30 minutes with or without the T1h 10 ug/ml and hR3 which is used as nonspecific control in a sterile BD FACS 5 ml tube. After incubation, cells were vortexed and 100 ≈l of the cell suspension was added to the respective wells. 100 ul of the Tetanus toxoid (Cat #582231, CALBIOCHEM) (10 ug/ml) working solution (RPMI media with 5% FBS) was added to the respective wells to stimulate the memory T-cell proliferation. The plates were incubated for five days in the CO_2 incubator at 37éC. 65 ≈l of Alamar blue was added to each well and incubated overnight in a CO_2 incubator at 37éC. Fluorescence was measured on a spectrophotometer Biotek Synergy.TM. HT with 530 nm excitation, 590 nm emission, and sensitivity=35.

The experimental results as set forth in Figure 12 show that Tetanus Toxoid does stimulate the proliferation of T-cells in a dose dependent manner, but the T1h does not show any inhibition of proliferation of these cells. This strongly suggests that T1h does not inhibit memory T-cell proliferation. This is favorable for T1h therapy because circulating memory T-cell proliferation is not affected and patients on T1h therapy would not become susceptible to infection.

Example 7

T1h Inhibits T-cell Proliferation in a Mixed Lymphocyte Reaction Mediated by PBMCs and Raji Cells

Raji/PBMCs cells were harvested and resuspended in 1xPBS. 8 x 10^5 cells/ml of Raji cells/PBMCs were resuspended in 1 ml of mitomycin (25 ug/ml). Cells were incubated for 30 minutes in a CO_2 incubator at 37éC. After Incubation 2 ml of RPMI with 5% FBS was added to each tube and centrifuged at 1200 RPM for 5 minutes at RT to remove the mitomycin. The supernatant was discarded and again 2 ml of RPMI with 5 FBS was added and centrifuged. Supernatant was discarded and cells are resuspended in the RPMI media

50 ul of PBMCs (4 x 10^5 cells/ml) was added to the respective wells of 96 well round bottom plates. 100 ≈l of antibody dilution T1h or hR3 (10 ug/ml) was added to the respective wells and incubated for 30 minutes in a CO_2 incubator at 37éC. 50 ul of the Mitomycin treated Raji cells (4 x 10^5 cells/ml) was added into the respective wells. Along with the assay, controls which were included were Mitomycin treated Raji cells alone, PBMCs alone, Mitomycin treated Raji cells+PHA, PBMCs+PHA, Mitomycin treated Raji and PBMCs. The plate was incubated for 5 days in a CO_2 incubator at 37éC. 65 ≈l of Alamar blue was added to each well and incubated overnight in a CO_2 incubator at 37éC. Fluorescence was measured on a spectrophotometer Biotek Synergy.TM. HT with 530 nm excitation, 590 nm emission, and sensitivity=35. In conclusion it was observed, see the results of Figures 13 and 14, that T1h can specifically inhibit one way MLR where Raji cells are the Antigen Presenting Cells and PBMCs.

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Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

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The contents of all references cited herein are hereby incorporated by reference herein for all purposes.

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EP 0807125

PCT/IN2008/00562

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CLAIMS

1. A composition for treating lupus in a subject comprising an anti-CD6 monoclonal antibody (T1h) that binds to domain 1 (D1) of CD6 without interfering with ALCAM binding to CD6, wherein the anti-CD6 monoclonal antibody comprises amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2, and wherein the anti-CD6 monoclonal antibody is in a therapeutically effective amount to reduce the symptoms of lupus in the subject.

2. The composition of claim 1, wherein lupus comprises systemic lupus erythematosus, lupus nephritis, cutaneous lupus erythematosus, central nervous system (CNS) lupus, neonatal lupus erythematosus, childhood systemic lupus erythematosus, drug- induced lupus erythematosus or complement deficiency syndromes resulting in lupus manifestations.

3. The composition of claim 1, wherein the symptoms of lupus comprise cardiovascular manifestations, pulmonary manifestations, hepatic manifestations, haematologieal manifestations, gastrointestinal manifestations and/or musculoskeletal manifestations.

4. The composition of claim 1, wherein the anti-CD6 monoclonal antibody reduces or prevents the activation of T-cells, inhibits T-cell proliferation and/or reduces induction of complement-dependent cytotoxicity (CDC).

5. The composition of claim 1, wherein the anti-CD6 monoclonal antibody is encoded by a nucleotide sequence comprising SEQ ID NO: 3 and SEQ ID NO: 4 or nucleotide sequences have at least 90% identity thereto and encodes for SEQ ID NO: 1 and SEQ ID NO: 2.

6. The composition of claim 1, formulated for delivery by parenteral administration.

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7. The composition of claim 1, further comprising a pharmaceutically acceptable carrier.

8. The composition of claim 1, wherein the anti-CD6 monoclonal antibody is combined with a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial drug, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

9. The composition of claim 8, wherein the immunosuppressant is prednisone, methotrexate, azathioprine or cyclophosphamide.

10. The composition of claim 1, wherein the therapeutically effective amount is about 0.01 to about 100 mg/kg per subject body weight.

11. The composition of claim 1, wherein the anti-CD6 monoclonal antibody causes a reduction in pro inflammatory cytokines.

12. A method for treating lupus in a subject comprising administering an anti-CD6 monoclonal antibody (T1h) that binds to domain 1 (D1) of CD6 without interfering with ALCAM binding to CD6, wherein the anti-CD6 monoclonal antibody comprises amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2, and wherein the anti-CD6 monoclonal antibody is in a therapeutically effective amount to reduce the symptoms of lupus in the treated subject.

13. The method of claim 12, wherein lupus comprises systemic lupus erythematosus, lupus nephritis, cutaneous lupus erythematosus, central nervous system (CNS) lupus, neonatal lupus erythematosus, childhood systemic lupus erythematosus, drug- induced lupus erythematosus or complement deficiency syndromes resulting in lupus manifestations.

14. The method of claim 12, wherein the symptoms of lupus comprise cardiovascular manifestations, pulmonary manifestations, hepatic manifestations, haematologieal manifestations, gastrointestinal manifestations and/or musculoskeletal manifestations.

15. The method of claim 12, wherein the anti-CD6 monoclonal antibody reduces or prevents the activation of T-cells, inhibits T-cell proliferation and/or reduces induction of complement-dependent cytotoxicity (CDC).

16. The method of claim 12, wherein the anti-CD6 monoclonal antibody is encoded by a nucleotide sequence comprising SEQ ID NO: 3 and SEQ ID NO: 4 or nucleotide sequences have at least 90% identity thereto and encodes for SEQ ID NO: 1 and SEQ ID NO: 2.

17. The method of claim 12, wherein the anti-CD6 monoclonal antibody is administered by parenteral delivery.

18. The method of claim 12, further comprising combining the anti-CD6 monoclonal antibody with a pharmaceutically acceptable carrier.

19. The method of claim 12, wherein the anti-CD6 monoclonal antibody is combined with a chemotherapeutic agent, an immunosuppressive agent, an anti-malarial drug, a cytotoxic agent, an integrin antagonist, a cytokine antagonist, or a hormone.

20. The composition of claim 12, wherein the therapeutically effective amount is about 0.01 to about 100 mg/kg per subject body weight.

21. The method of claim 12, wherein the anti-CD6 monoclonal antibody causes a reduction in pro inflammatory cytokines.

22. The method of claim 12, wherein the subject does not have an autoimmune disease other than lupus.

23. A method to reduce activation of T-cells in a subject patient with an elevated level of anti- nuclear antibodies (ANA) and/or anti-double-stranded DNA (dsDNA) antibodies, the method comprising administering to the subject a therapeutically effective amount of

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an anti-CD6 monoclonal antibody, wherein the anti-CD6 monoclonal antibody comprises amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

24. The method of claim 23, wherein the therapeutically effective amount is about 0.01 to about 100 mg/kg per subject body weight.

25. Use of an anti-CD6 monoclonal antibody in the manufacture of medicament useful for the treatment of lupus, wherein the wherein the anti-CD6 monoclonal antibody comprises amino acid sequences of SEQ ID NO: 1 and SEQ ID NO: 2.

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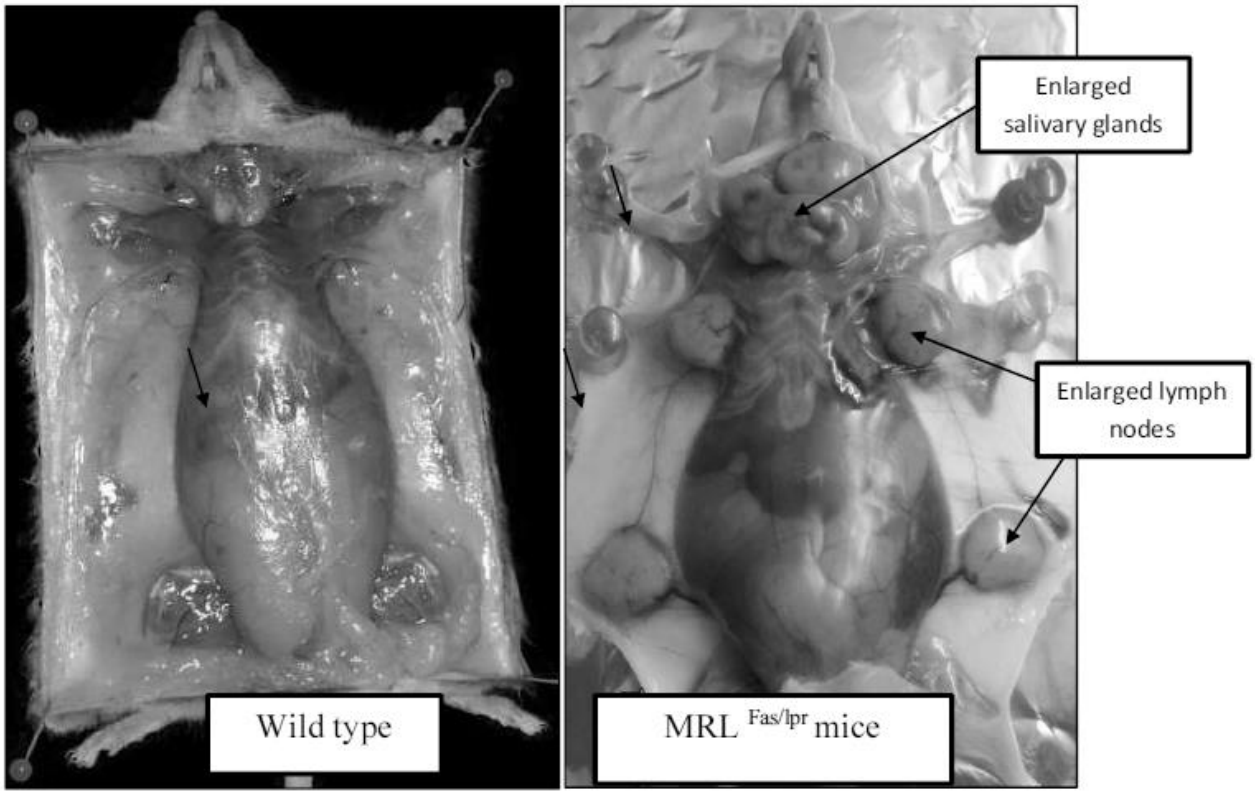

Figure 1

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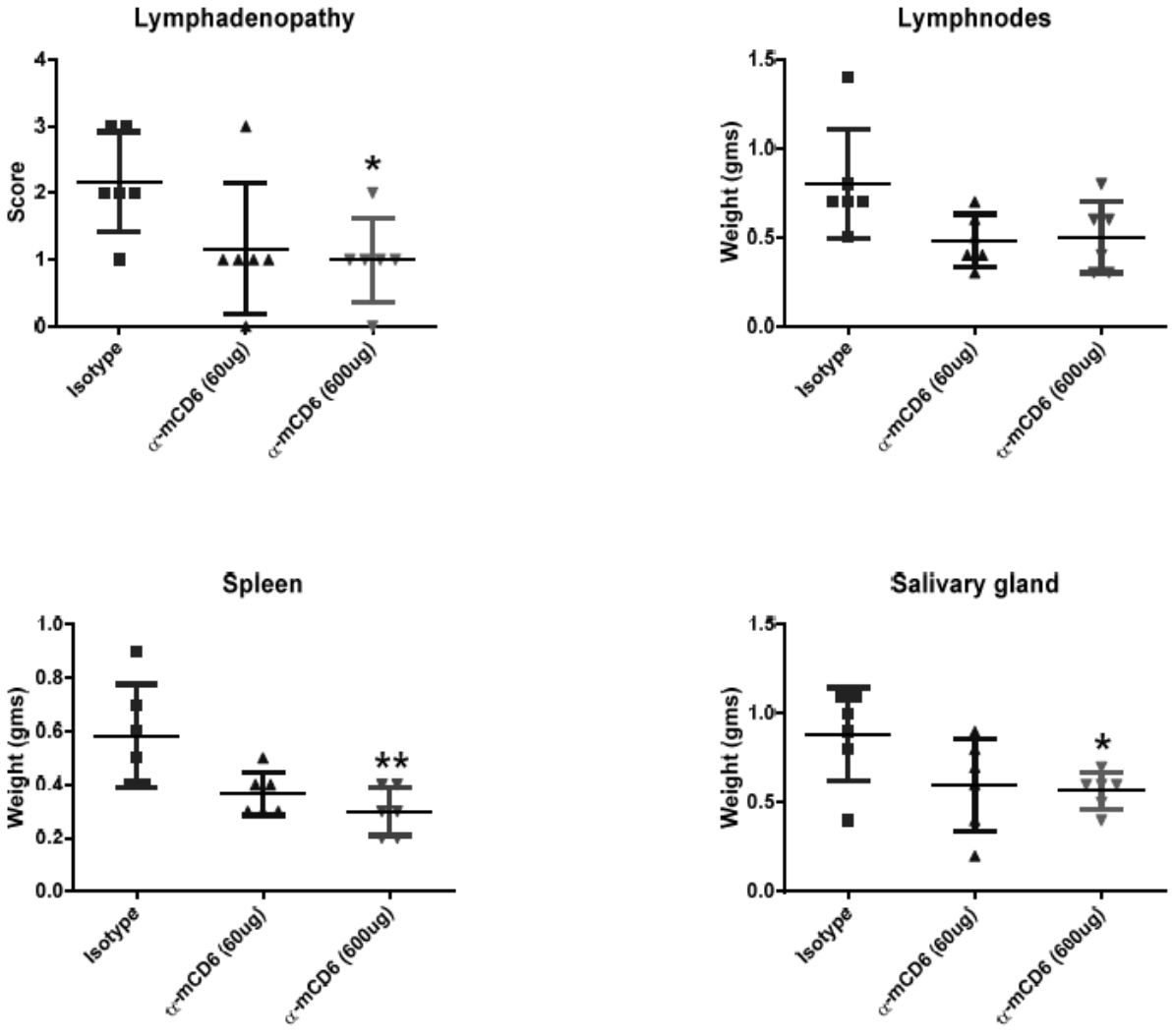

Figure 2

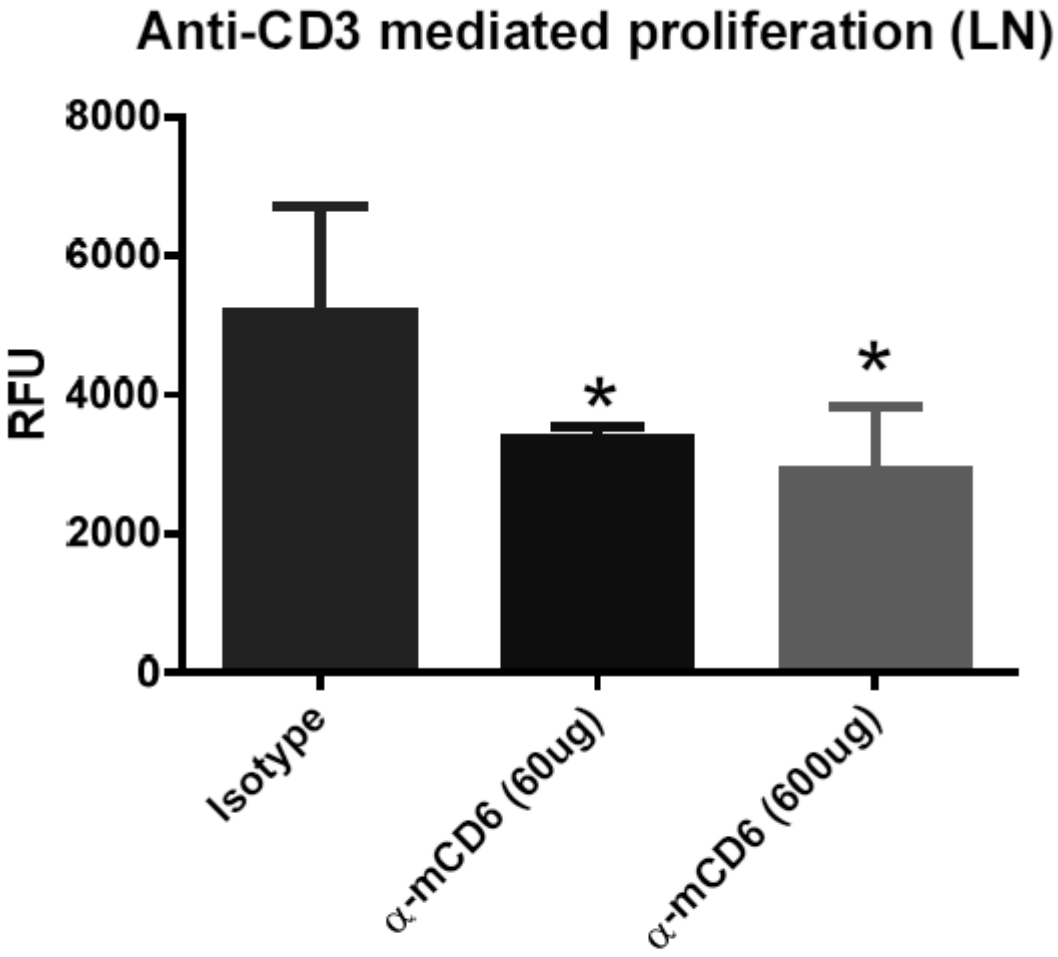

Figure 3

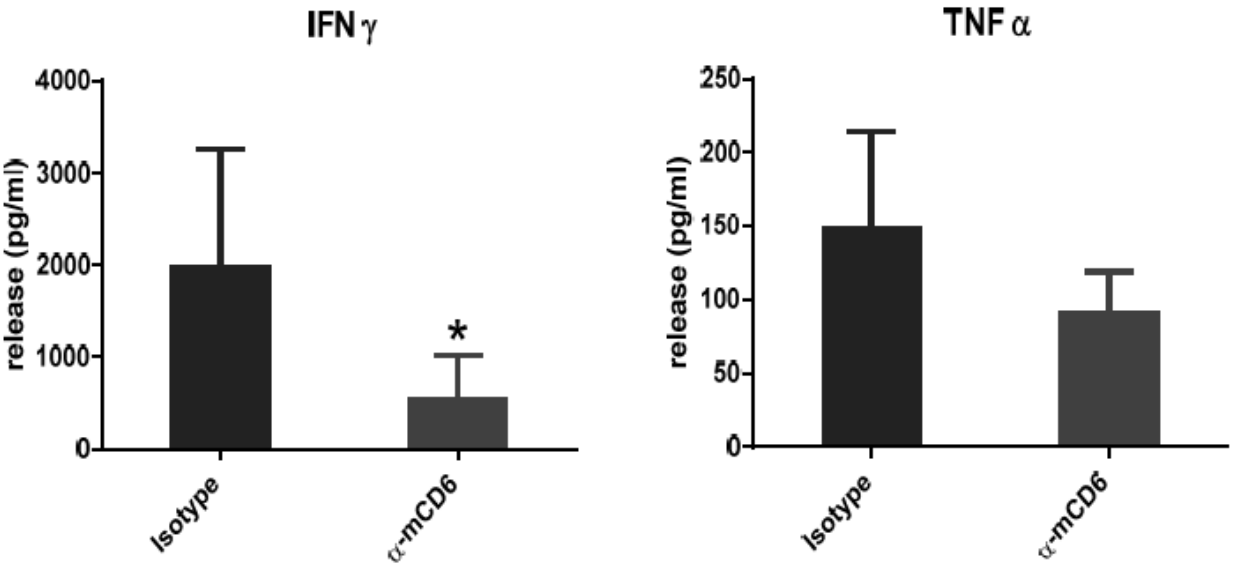

Figure 4

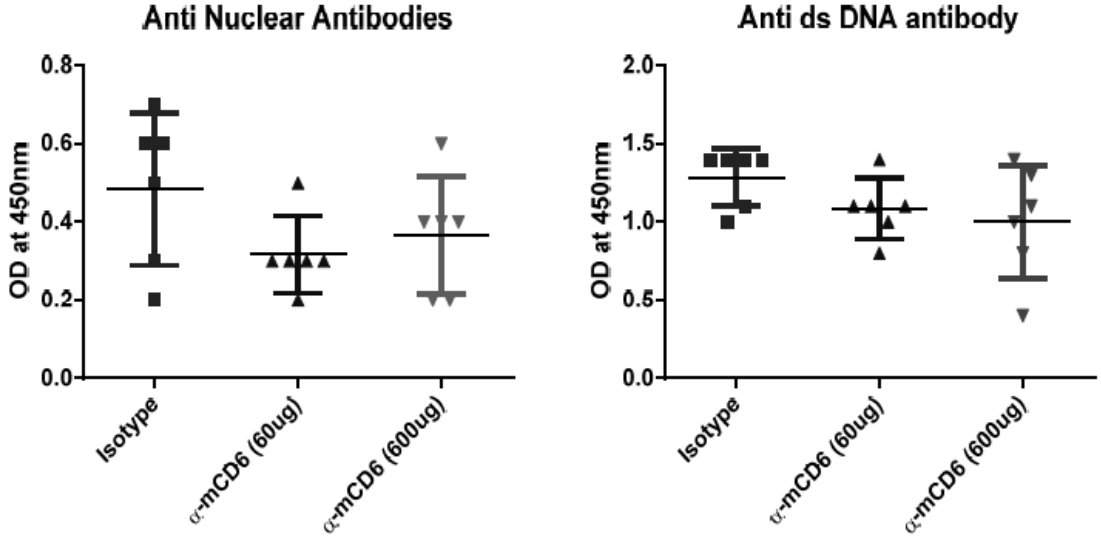

Figure 5

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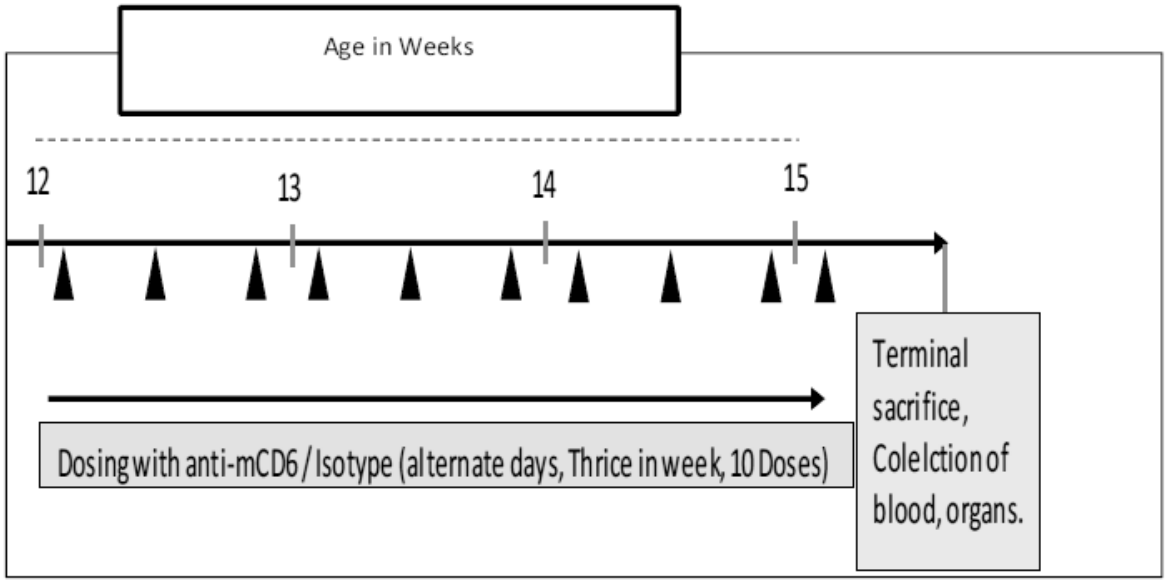

Figure 6

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VH sequence:

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGG
TCCCTGAAACTCTCCTGTGCAGCCTCTGGATTCAAGTTTAGTAGATATG
CCATGTCTTGGGTTCGCCAGGCTCCGGGGAAGAGGCTGGAGTGGGTCG
CAACCATTAGTAGTGGTGGTAGTTACATCTACTATCCAGACAGTGTGAA
GGGTCGATTCACCATCTCCAGAGACAATGTCAAGAACACCCTGTATCTG
CAAATGAGCAGTCTGAGGTCTGAGGACACGGCCATGTATTACTGTGCA
AGACGAGATTACGACCTGGACTACTTTGACTCCTGGGGCCAAGGCACC
CTTGTCACCGTCTCCTCA

Vk sequence:

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCGGTGGGAG
ACAGAGTCACTATCACTTGCAAGGCGAGTCGGGACATTAGAAGCTATT
TAACCTGGTACCAGCAGAAACCAGGGAAAGCTCCTAAGACCCTGATCT
ATTATGCAACAAGCTTGGCAGATGGGGTCCCGTCGAGATTCAGTGGCA
GTGGATCTGGGCAAGATTATTCTCTCACCATCAGCAGCCTGGAGTCTGA
CGATACAGCAACTTACTACTGTCTACAACATGGTGAGAGTCCATTCACG
CTCGGCTCGGGGACCAAGCTGGAAATCAAA

Figure 7A

VH sequence:

E V Q L V E S G G G L V K P G G S L K L S C A A S G F K F S R Y A M S W
V R Q A P G K R L E W V A T I S S G G S Y I Y Y P D S V K G R F T I S R D
N V K N T L Y L Q M S S L R S E D T A M Y Y C A R R D Y D L D Y F D S
W G Q G T L V T V S S

VK sequence:

D I Q M T Q S P S S L S A S V G D R V T I T C K A S R D I R S Y L T W Y Q
Q K P G K A P K T L I Y Y A T S L A D G V P S R F S G S G S G Q D Y S L T
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Figure 7B

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Figure 7C

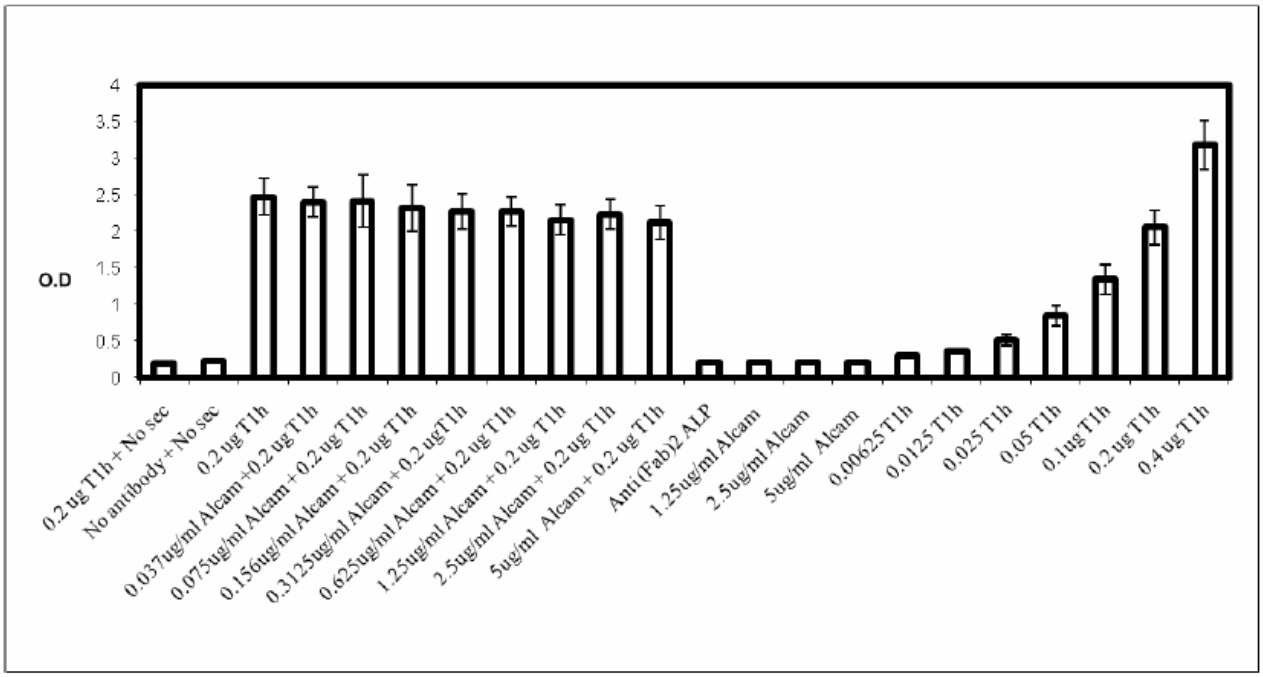


Figure 8

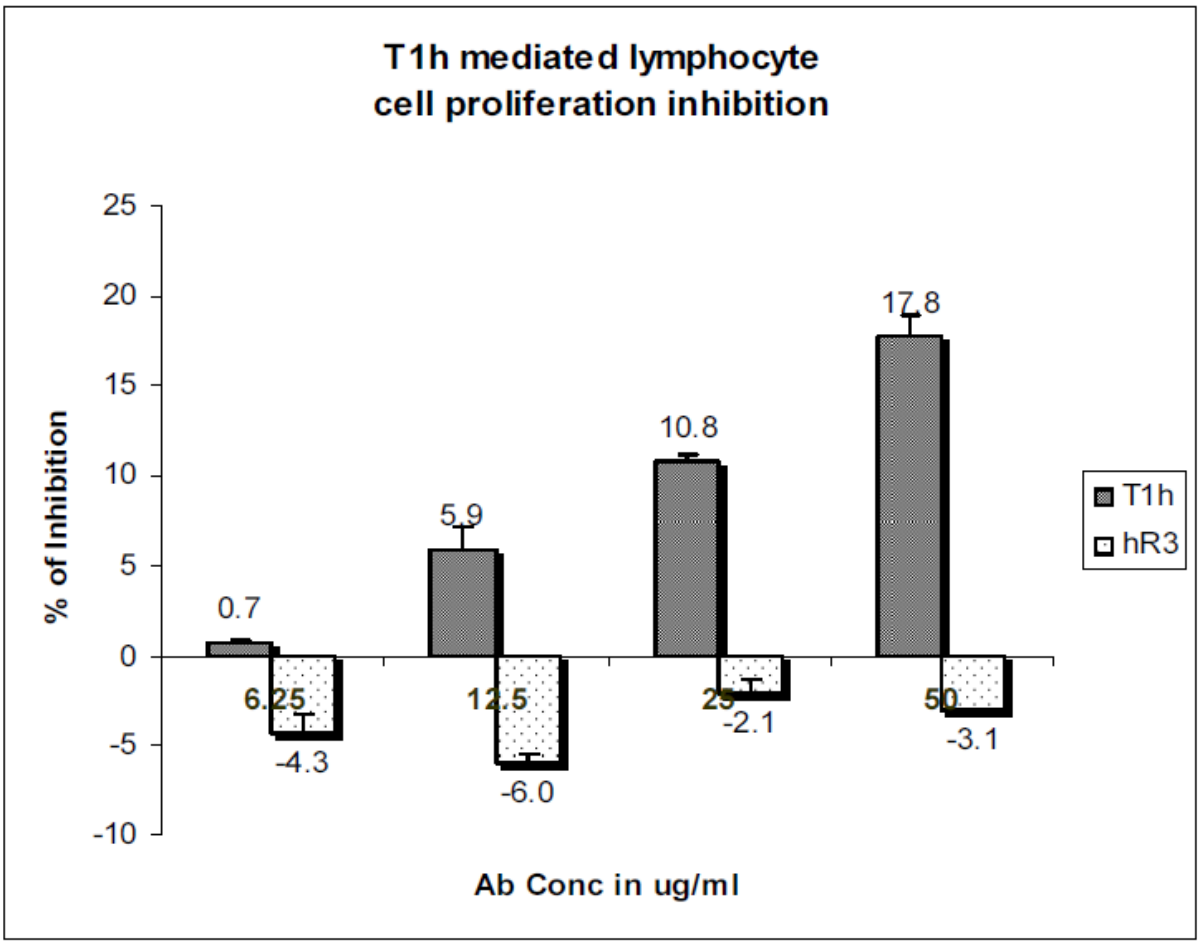


Figure 9

2024227640 25 Oct 2024

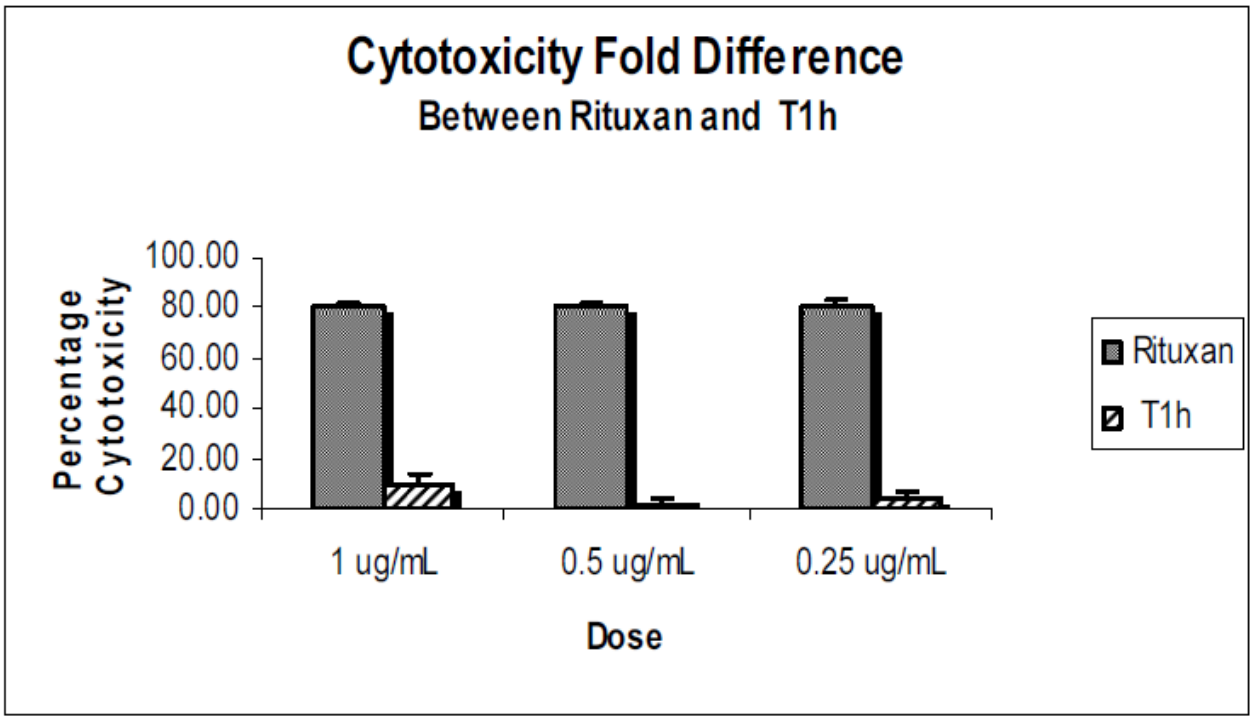
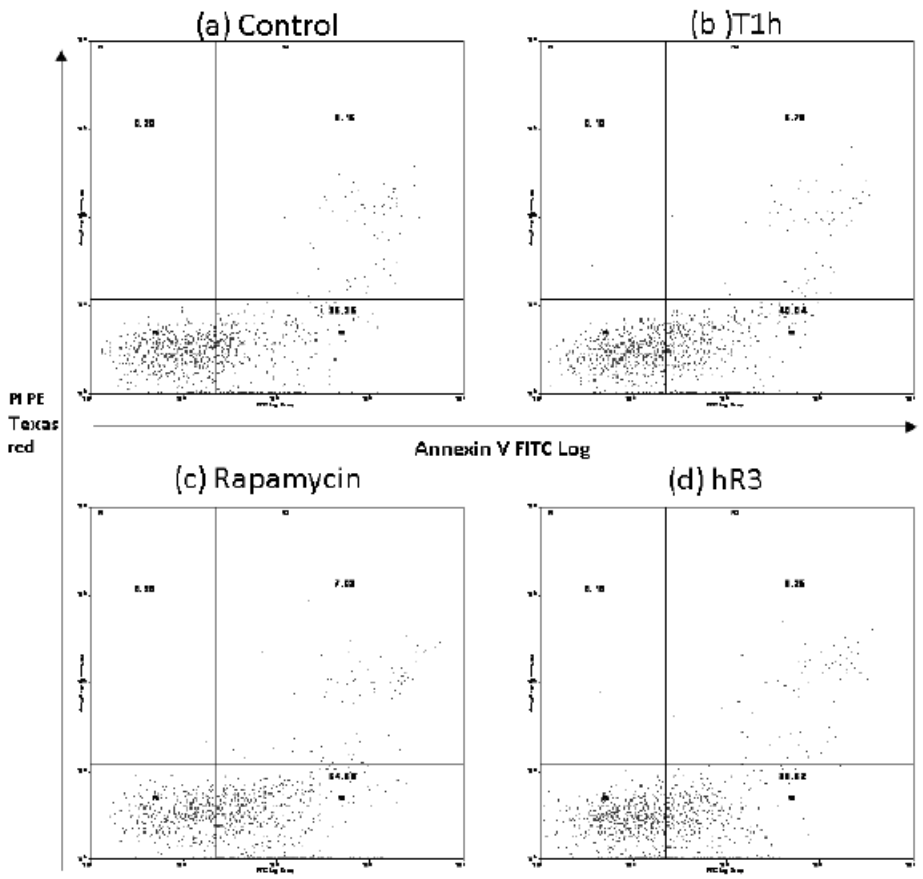


Figure 10



Annexin v positive	
Control	35.3%
T1h (5ug/ml)	40.0%
Rapamycin (1.2ug/ml)	54.3%
hR3 (5ug/ml)	36.5%

Figure 11

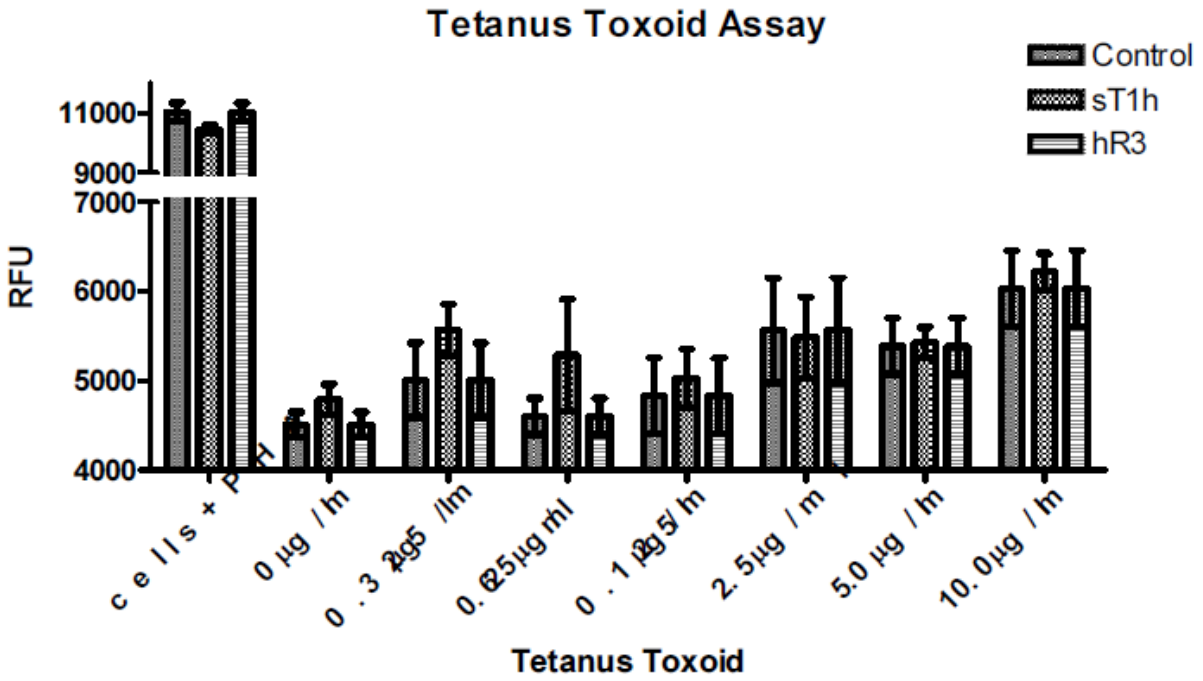


Figure 12

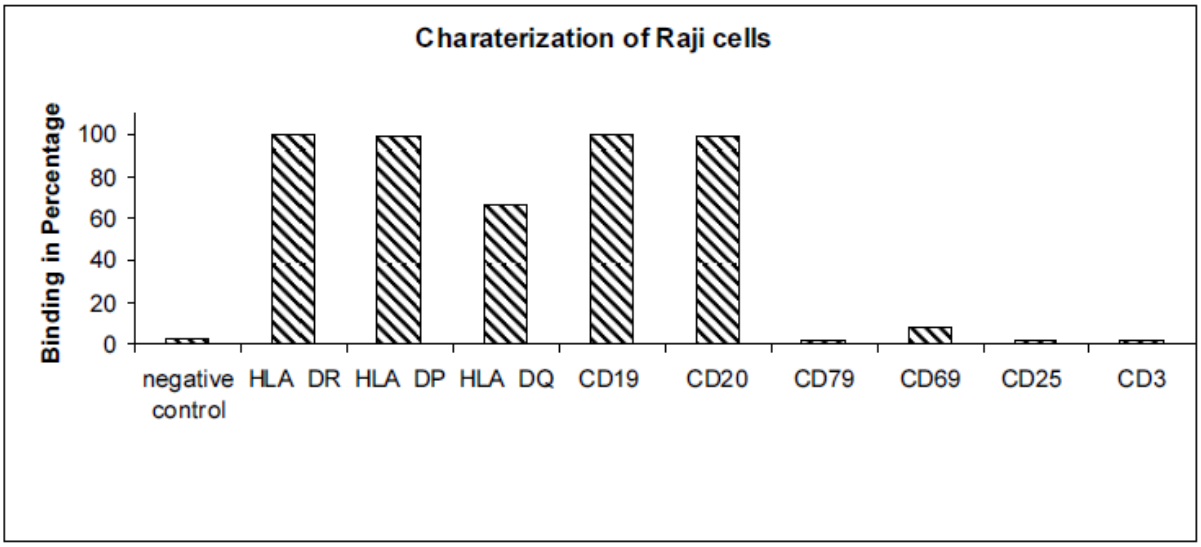


Figure 13

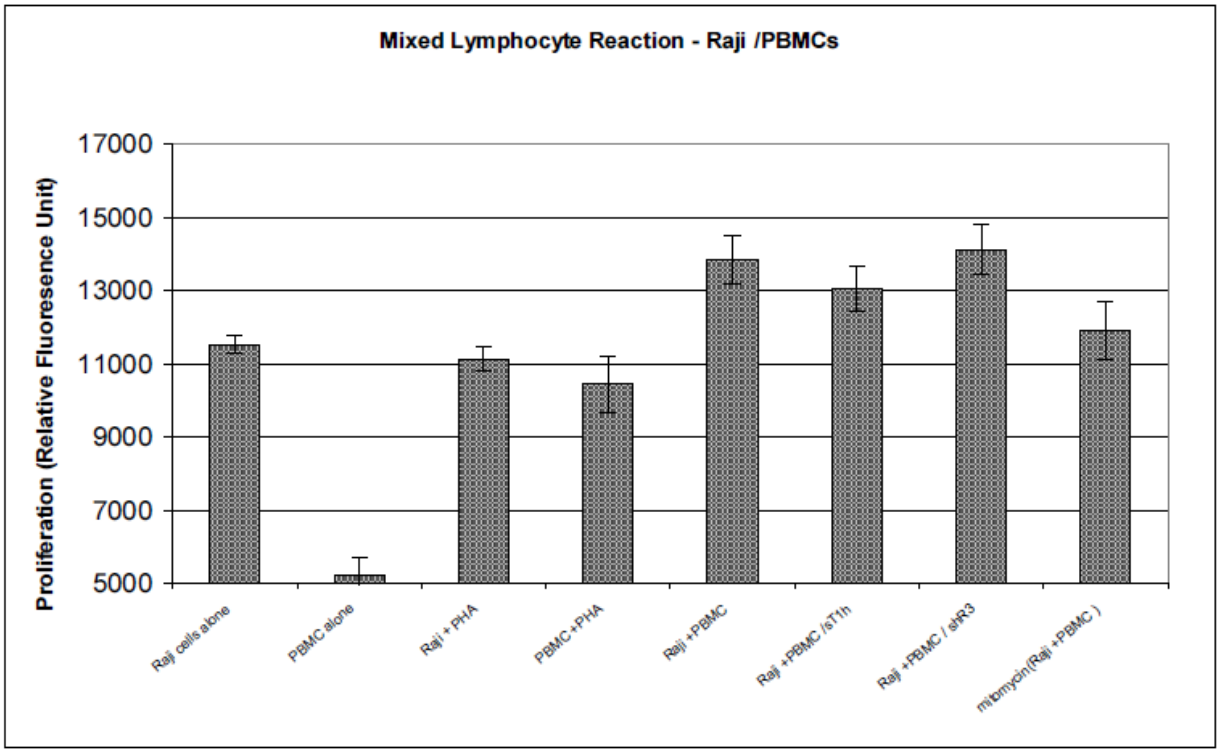


Figure 14

Sequence Listing

1	**Sequence Listing Information**	
1-1	File Name	35623403Biocon div.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.1.2
1-5	Production Date	2024-10-25
1-6	Original free text language code	en
1-7	Non English free text language code	
2	**General Information**	
2-1	Current application: IP Office	
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	35623403-TKU
2-5	Earliest priority application: IP Office	IN
2-6	Earliest priority application: Application number	201641036145
2-7	Earliest priority application: Filing date	2016-10-21
2-8en	Applicant name	Biocon Limited
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	A monoclonal antibody and a method of use for the treatment of lupus
2-11	Sequence Total Quantity	5

3-1	**Sequences**	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	AA
3-1-3	Length	119
3-1-4	Features	**REGION 1..119**
	Location/Qualifiers	note=Synthetic Construct
		source 1..119
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-1-5	Residues	EVQLVESGGG LVKPGGSLKL SCAASGFKFS RYAMSWVRQA PGKRLEWVAT ISSGGSYIYY 60 PDSVKGRFTI SRDNVKNTLY LQMSSLRSED TAMYYCARRD YDLDYFDSWG QGTLVTVSS 119
3-2	**Sequences**	
3-2-1	Sequence Number [ID]	2
3-2-2	Molecule Type	AA
3-2-3	Length	107
3-2-4	Features	**REGION 1..107**
	Location/Qualifiers	note=Synthetic Construct
		source 1..107
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-2-5	Residues	DIQMTQSPSS LSASVGDRVT ITCKASRDIR SYLTWYQQKP GKAPKTLIYY ATSLADGVPS 60 RFSGSGSGQD YSLTISSLES DDTATYYCLQ HGESPFTLGS GTKLEIK 107
3-3	**Sequences**	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	DNA
3-3-3	Length	357
3-3-4	Features	**misc_feature 1..357**
	Location/Qualifiers	note=Synthetic construct
		exon 1..357
		source 1..357
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-3-5	Residues	gaagtgcagc tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaaactc 60 tcctgtgcag cctctggatt caagtttagt agatatgcca tgtcttgggt tcgccaggct 120 ccggggaaga ggctggagtg ggtcgcaacc attagtagtg gtggtagtta catctactat 180 ccagacagtg tgaagggtcg attcaccatc tccagagaca atgtcaagaa caccctgtat 240 ctgcaaatga gcagtctgag gtctgaggac acggccatgt attactgtgc aagacgagat 300 tacgacctgg actactttga ctcctggggc caaggcaccc ttgtcaccgt ctcctca 357
3-4	**Sequences**	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	DNA
3-4-3	Length	318
3-4-4	Features	**misc_feature 1..318**
	Location/Qualifiers	note=Synthetic Construct
		exon 1..318
		source 1..318
		mol_type=other DNA
		organism=synthetic construct
	NonEnglishQualifier Value	
3-4-5	Residues	gacatccaga tgacccagtc tccatcctcc ctgtctgcat cggtgggaga cagagtcact 60 atcacttgca aggcgagtcg ggacattaga agctatttaa cctggtacca gcagaaacca 120 gggaaagctc ctaagaccct gatctattat gcaacaagct tggcagatgg ggtcccgtcg 180 agattcagtg gcagtggatc tgggcaagat tattctctca ccatcagcag cctggagtct 240 gacgatacag caacttacta ctgtctacaa catggtgaga gtccaacgct cggctcgggg 300 accaagctgg aaatcaaa 318
3-5	**Sequences**	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	AA
3-5-3	Length	109
3-5-4	Features	**REGION 1..109**
	Location/Qualifiers	note=Synthetic Construct
		source 1..109
		mol_type=protein
		organism=synthetic construct
	NonEnglishQualifier Value	
3-5-5	Residues	DIQMTQSPSS LSASVGDRVT ITCKASRDIR SYLTWYQQKP GKAPKTLIYY ATSLADGVPS 60 RFSGSGSGQD YSLTISSLES DDTATYYCLQ HGESPFTFGS GTKLEIKRA 109