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(54) Title: IMPROVEMENTS TO $\gamma\delta$ T CELL-MEDIATED THERAPY

(57) Abstract: The present invention relates to compositions and methods for determining or modulating the activity of immune and/or target cells in vitro, ex vivo or in vivo. The invention more specifically relates to methods and compositions for determining sensitivity of cells to the activity of $\gamma\delta$ T cells, as well as to the use of these compositions and methods for patient screening or selection, therapy improvement, compound selection, etc. The invention also provides compositions and kits suitable for carrying out these methods. The methods may be used in any mammalian subject, preferably any human subject that may benefit from $\gamma\delta$ T cell-mediated therapy, including patients with tumors, immune or infectious diseases.



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Improvements to $\gamma\delta$ T cell-mediated therapy

The present invention relates to compositions and methods for determining or modulating the activity of immune and/or target cells in vitro, ex vivo or in vivo. The invention more specifically relates to methods and compositions for determining sensitivity of cells to the activity of $\gamma\delta$ T cells, as well as to the use of these compositions and methods for patient screening or selection, therapy improvement, compound selection, etc. The invention also provides compositions and kits suitable for carrying out these methods. The methods may be used in any mammalian subject, preferably any human subject that may benefit from $\gamma\delta$ T cell-mediated therapy, including patients with tumors, immune or infectious diseases.

INTRODUCTION

Peripheral T lymphocytes classically recognize, through their $\alpha\beta$ T cell receptors (TCR), foreign peptidic antigens bound to class I or class II major histocompatibility complex (MHC) molecules. Besides these "conventional" T cells, other subsets expressing either $\alpha\beta$ or $\gamma\delta$ TCR react with a more heterogeneous set of non-peptidic compounds, either in a native form or in association with conserved MHC-related molecules (Beckman et al., 1994 ; Moody et al., 1997 ; Spada et al., 2000).

In humans, the vast majority of peripheral blood $\gamma\delta$ T cells use a particular combination of variable regions (V γ 9 and V δ 2) to form their TCR. These V γ 9V δ 2 T cells are activated in a TCR-dependent fashion by several small phosphorylated (Constant et al., 1994 ; Tanaka et al., 1994) or aminated (Bukowski et al., 1999) alkyl molecules. V γ 9V δ 2 T cell activation by these compounds requires intercellular contact, thus suggesting some form of antigen presentation (Lang et al., 1995 ; Morita et al., 1995). V γ 9V δ 2 cells also react against several fresh or cultured tumors in vitro and exhibit both cytolytic activity and production of inflammatory cytokines (TNF α , IFN γ). This activity is tightly regulated by NK-like receptors for MHC class-Ia and class Ib antigens which are prominently expressed by this T cell subset (Fisch et al., 1997 ; Halary et al., 1997). Thus, besides their role in immunity against viral and bacterial infections (Bukowski et al., 1994), $\gamma\delta$ T cells are probably involved in tumor surveillance (Bukowski et al., 1995 ; Fisch et al., 1997 ; Wu et al., 2002) as also supported by in vivo experiments (Girardi et al., 2001; Malkovska et al., 1994 ; Malkovska et al., 1992).

Although some human $\gamma\delta$ T cells of the V δ 1 subset react towards non classical, stress-induced MHC molecules MICA/B (Groh et al., 1998 ; Wu et al., 2002) through their TCR and/or activatory receptors such as NKG2D, tumor antigens recognized by V γ 9V δ 2 T cells remain unknown and strategies for activating these cells, as well as for screening target

cells sensitive to $\gamma\delta$ T cells' activity are highly needed to develop powerful therapeutic strategies.

SUMMARY OF THE INVENTION

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The present invention now discloses novel, improved strategies for generating, regulating or exploiting the activity of $\gamma\delta$ T cells. The present invention unexpectedly shows that an entity related to the mitochondrial ATP-synthase is expressed on the membrane of target tumor cells and promotes their recognition by V γ 9V δ 2 T cells. When immobilized, purified
10 ATP synthase induces the selective activation of this cell population. The V γ 9V δ 2 TCR and the ATP synthase also bind a delipidated form of apolipoprotein A-I as demonstrated by surface plasmon resonance. Moreover, the presence of apolipoprotein A-I in the culture medium is required for optimal activation of V γ 9V δ 2 T cells and TCR binding to tumors
15 expressing ATP synthase. This invention thus identifies an unanticipated tumor recognition mechanism by V γ 9V δ 2 lymphocytes and allows the design of novel therapeutic and pharmacogenomic approaches.

A first aspect of this invention resides more particularly in methods of assessing the sensitivity of a pathologic cell to $\gamma\delta$ T cell activity (e.g., lysis or cytotoxicity), comprising
20 determining in vitro or ex vivo whether said pathologic cell expresses, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being an indication of the sensitivity of the pathologic cell to $\gamma\delta$ T cell lysis or cytotoxicity.

25 An other aspect of this invention resides in methods of assessing whether a cell is a target cell for $\gamma\delta$ T cells, comprising determining in vitro or ex vivo whether said cell expresses, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being an indication that the cell is a target of $\gamma\delta$ T cells.

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A further aspect of this invention lies in methods of assessing the responsiveness of a patient to $\gamma\delta$ T cell-mediated therapy, comprising determining whether said patient contains pathologic cells, tissues or organs that express, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase
35 polypeptide or a portion thereof being an indication of the responsiveness of the patient to $\gamma\delta$ T cell-mediated therapy.

The invention also relates to methods of selecting patients for a $\gamma\delta$ T cell-mediated therapy program, comprising determining whether a patient contains pathologic cells, tissues or

organs that express, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being a criteria for selecting said patient for said $\gamma\delta$ T cell-mediated therapy program.

5 A further object of this invention resides in methods of stimulating $\gamma\delta$ T cells, comprising contacting $\gamma\delta$ T cells with an ATP synthase polypeptide or a TCR-binding fragment thereof. In a specific embodiment, the stimulation method uses a complex comprising an ATP synthase polypeptide or a TCR-binding fragment thereof linked to an apolipoprotein A1 polypeptide. These components (or some of them) may be immobilized on a solid support,
10 such as a bead, plate, etc.

A further object of this invention resides in methods of stimulating $\gamma\delta$ T cells, comprising contacting $\gamma\delta$ T cells with an apolipoprotein A1 polypeptide or a variant or ATP synthase-binding fragment thereof.

15 The invention also provides a method of increasing sensitivity of a target cell to $\gamma\delta$ T cell lysis or cytotoxicity, comprising causing or increasing cell surface expression of an ATP synthase polypeptide or a portion thereof in said target cell. The method may be performed in vitro, ex vivo or in vivo.

20 The invention also relates to an improvement in methods of treating a cancer, an infectious disease, an autoimmune disease or an allergic disease in a subject by $\gamma\delta$ T cell-mediated therapy, the improvement comprising determining, prior to or during said therapy, whether said subject contains pathologic cells, tissues or organs that express, at the surface thereof,
25 an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being a criteria for selecting or monitoring said patient for said $\gamma\delta$ T cell-mediated therapy.

The invention also relates to methods of screening, selecting or identifying a compound,
30 comprising:

- a) separately contacting $\gamma\delta$ T cells with an ATP synthase polypeptide or a TCR-binding fragment thereof in the presence and in the absence of a candidate compound and,
- b) screening, selecting or identifying a compound that modulates activation of said $\gamma\delta$ T cells by said ATP synthase polypeptide or a TCR-binding fragment thereof.

35 The invention is particularly suited for use with human cells or subjects, and can be used to diagnose or select cells or patients having cancers or infectious diseases sensitive to $\gamma\delta$ T cell-mediated therapy. As will be discussed further, the ATP synthase polypeptide may be

an ATP synthase α subunit or β subunit or both, and the portion thereof may be an extracellular domain of an ATP synthase α or β subunit.

LEGEND TO THE FIGURES

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Figure 1: Binding of extracellular apoA-I to tumor cell membrane.

(a) Indirect immunofluorescence staining with M5 mAb or a control IgG1 antibody (shaded histograms) of human cell lines cultured in presence of fetal calf serum (fcs). Daudi, Raji: Burkitt's lymphomas; Awells, RPMI8866: B-lymphoblastoid cell lines; RPMI 8226: B-cell myeloma; K562: erythroid leukemia; U937: monocytic leukemia; MOLT-4, Jurkat: T cell leukemias.

(b) Daudi cells were depleted of serum components by overnight culture in serum free medium (sfm) and subsequently incubated in PBS supplemented with either 10% fcs (sfm/fcs), 10% human serum (sfm/hs) or 1% BSA plus 100 μ g/ml of M5L (sfm/M5L) before staining as in figure 1a.

(c) Serum-depleted cell lines were incubated with lipid-free HDL-apoA-I (solid lines and shaded histograms) or BSA (dotted lines) before indirect staining with the anti-human apoA-I antibody 4H1 (lines) or control IgG1 (shaded histograms).

(d) Left: coomassie-blue staining of M5-immunopurified material from fcs (lane 1) and human serum (lane 2) after non-reducing SDS-polyacrylamide gel electrophoresis (PAGE). Right: Immunoblot after reducing PAGE using anti-human apoA-I antibody 4H1 on : human serum (lane 3); fetal calf serum (lane 4); HDL (lane 5); hM5L (human serum ligand of M5 mAb, lane 6).

(e): ^{125} I-labelled free apoA-I binding was measured after 2 hours incubation at 4°C on Daudi and Raji cells. Top and bottom panels: binding isotherm of ^{125} I-labeled free apoA-I to Daudi (top) and Raji (bottom) cells. (○): total binding; (σ): non-specific binding; (●): specific binding. Middle panel: Scatchard representation of specific binding on Daudi cells. In absence of specific binding on Raji cells, Scatchard representation could only be performed from Daudi cells data. The results are representative of two different cell preparations.

Figure 2 : ApoA-I-dependent activation of V γ 9V δ 2 T cells. ^{51} Cr release assays were performed in serum-free medium (sfm) to assess dependance on serum and apoA-I of Daudi cytotoxicity by different effector T cell populations. G25 and G42: V γ 9V δ 2 T cell clones, 73R9: V γ 8V δ 3 T cell clone. M5L (bovine serum component immunopurified with M5 antibody) was tested at the indicated concentration for its ability to substitute for serum (fcs). Recombinant annexin V was used as a control purified protein.

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Figure 3 : V γ 9V δ 2 TCR binding to tumor cells and apoA-I. PE-labeled tetrameric TCRs from clone G115 and clone 73R9 were used to stain tumor cell lines and assess the influence of apolipoproteins on their tumor recognition. Shaded histograms: concentration-matched streptavidin-PE alone. Line histograms: binding of the indicated PE-labelled tetrameric TCR. (a) Cell lines were cultured in 10% fcs before staining with tetramers. (b) K562 cells were serum-depleted, incubated with 10 % human serum or the indicated apolipoprotein preparations (100 μ g/ml) and stained with G115 or 73R9 TCR tetramers. (c) Serum-depleted K562 cells were incubated with 100 μ g/ml of HDL hApoA-I (left histogram) or hM5L (right histogram) and labeled with G115 TCR tetramers in the presence of the indicated competing mAb (20 μ g/ml). Note that M5 does not recognize HDL hApoA-I (see text) whereas 4H1 recognizes both forms of the human protein. Data are representative of three experiments.

Figure 4 : Expression of ATP-synthase-related structures on tumor cells. (a,b) Indirect immunofluorescence surface staining of haematopoietic tumor lines with (a) anti α -AS and (b) anti- β -AS. (c) Four kidney tumours sensitive to γ 9 δ 2 lysis were tested for expression of AS by fcs staining using control IgG (shaded histogram), anti- α -AS (dark line) and anti- β -AS (dotted line).

Figure 5 : ApoA-I and AS-dependent activation of V γ 9V δ 2 T cells. Daudi cells were incubated in serum, washed and incubated with serum-depleted V γ 9V δ 2 cells (clone G42) in sfm medium, in the presence of the indicated concentration of antibodies, and the production of γ -interferon was measured after a 20 hours co-culture. NaN₃-containing anti-AS and control antibodies were dialyzed before use (right panel). The phosphoantigen isopentenylpyrophosphate (IPP) was added in control cultures (2 μ g/ml) to exclude a possible toxicity of M5 and anti- β AS antibodies (left panel).

Figure 6 : Induction of V γ 9V δ 2 T cell lymphokine secretion by immobilized ATP-synthase. HDL-derived apoA-I and ATP synthase were immobilized on latex beads and these were used to stimulate T cell populations. none: no stimulation; empty: beads saturated with BSA; apoA-I: beads coated with apoA-I only. AS: beads coated with the F1 extra-membrane subunit of bovine ATP synthase. AS/apoA-I: beads coated with both protein preparations. (a) T cell clones were activated with protein-coated beads in medium supplemented with human serum, and TNF α secretion was measured in the culture supernatant after 4 hours. The capacity of $\gamma\delta$ clones G25, G115, 73R9 to secrete significant amounts of TNF α upon stimulation was checked by using Daudi cells as target cells as well as PMA/ionomycin or PHA. The $\alpha\beta$ clone A4.19 secretes saturating quantities of TNF α upon PMA/ionomycin or PHA stimulation (not shown).

(b) Stimulation was performed in the absence of exogenous serum (sfm) and purified HDL-derived apoA-I was added in some cultures (black bars).

(c) Fresh PBL were activated with indicated beads for 4 hours in the presence brefeldin A. Cells were then stained with antibodies to lymphocyte subsets, fixed, permeabilized and
 5 TNF α accumulation was analyzed after intracellular staining by flow cytometry. PMA/ionomycin stimulation is used as a control for TNF α -producing cells in the total population. Percentages indicating TNF α -producing cells within analysis quadrants and within each particular subset (between brackets) are shown.

10 **Figure 7. Surface plasmon resonance analysis.** Soluble proteins were exposed to the sensorchip surface for 240 s (association phase) followed by a 240-s flow running (dissociation phase). Immobilized proteins were on sensorchips flowcell 2. Sensorgrams are representative of specific interactions (differential response) where non-specific binding that occurred on flow cell 1 (with no protein immobilized) was deduced from binding that
 15 occurred on flow cell 2. Results are expressed as resonance units (RU) as a function of time in seconds.

(a,b) Overlay sensorgrams for SPR analysis of soluble TCR $\gamma\delta$ protein binding to immobilized apoA-I. Amount of immobilized ApoA-I protein was 2260 RU on flow cell 2
 20 (a): comparative sensorgrams of soluble V γ 9V δ 2TCR and V γ 8V δ 3TCR (200 and 2000nM) binding onto immobilized apoA-I. (b) V γ 9V δ 2TCR was injected at concentrations ranging from 125 nM to 2 μ M. The apparent kinetic constants of the interaction were $k_a=8.8e^3 \pm 6.08 e^2 M^{-1}s^{-1}$, $k_d=7.13 e^{-3} \pm 6.76 e^{-5} s^{-1}$, $K_D=8.1 e^{-7}M$.

(c) Comparative sensorgrams of purified apoA-I (full line) and apoA-II (dotted line) binding to immobilized V γ 9V δ 2TCR (proteins were injected at 100 μ g/ml). Amount of
 25 immobilized TCR $\gamma\delta$ protein was 335 RU on flow cell 2.

(d,e) Comparative sensorgrams of soluble TCR binding to immobilized ATP synthase. Amounts of immobilized ATP synthase (F1) was 22440 RU on flow cell 2. (d) Comparative sensorgrams of purified monomeric G115 TCR (V γ 9V δ 2, full line) and 73R9 TCR (V γ 8V δ 3, dotted line) binding to immobilized ATP synthase (F1). Proteins were
 30 injected at a concentration of 2 μ M. (e) Monomeric G115 soluble TCR (V γ 9V δ 2) was injected at concentrations ranging from 0.5 to 4 μ M. The apparent kinetic constants of the interaction were $k_a=1.68 e^3 \pm 2.16 M^{-1}s^{-1}$, $k_d=2.54 e^{-3} \pm 2.9 e^{-5} s^{-1}$, $K_D=1.51 e^{-6}M$.

DETAILED DESCRIPTION OF THE INVENTION

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T cells of the $\gamma\delta$ type are expressed by most mammalian species. They represent 1-10% of total circulating lymphocytes in healthy adult human subjects and most non-human primates. Most human peripheral blood $\gamma\delta$ T cells express a $\gamma\delta$ TCR heterodimer encoded by V γ 9/V δ 2 genes, some NK-lineage receptors for MHC class I and almost no CD4 nor

CD8. These cells have been shown to exhibit strong, non MHC-restricted, cytolytic activity against virus-infected cells (Poccia et al, J. Leukocyte Biology, 62, 1997, p. 1-5), parasite-infected cells (Constant et al, Infection and Immunity, vol. 63, n° 12, Dec. 1995, p. 4628-4633), or tumor cells (Fournie et Bonneville, Res. Immunol., 66th FORUM IN IMMUNOLOGY, 147, p. 338-347). These cells are also physiologically amplified in the context of several unrelated infectious diseases such as tuberculosis, malaria, tularemia, colibacillosis and also by B-cell tumors (for review see Hayday, 2000). These cells are thus viewed as potent effectors of innate immunity and represent an important resource of anti-infectious and anti-tumoral effectors. In this regard, $\gamma\delta$ T cell-mediated therapeutic programs are currently under development, particularly for treating cancers, using ex vivo activated cells or direct in vivo injection of $\gamma\delta$ T activators. However, in order to increase the efficacy of these treatments, it would be highly valuable to have methods available for selecting best responding patients or for selecting target cells, as well as for increasing the sensitivity of target or pathologic cells to these $\gamma\delta$ T cell-mediated therapies.

15

The instant invention now provides such methods.

As disclosed above, the invention relates to methods of assessing the sensitivity of cells to the activity of $\gamma\delta$ T cells, e.g., to their lysis or cytotoxicity. The tested cell may be any cell, preferably a mammalian cell, typically a human cell. The tested cell is generally a pathologic cell (i.e., a cell associated with or resulting from a disease in a subject), and the method is conducted in order to evaluate the efficacy of a $\gamma\delta$ T cell-mediated therapy against said pathology.

Typical examples of such target or test cells include pathogenic mammalian (e.g., human) tumor cells, pathogen-infected cells, pathologic immune cells, etc. Specific examples of tumor cells include any solid or hematopoietic tumor cell, such as renal cancer cells, liver cancer cells, lung cancer cells, bladder cancer cells, breast cancer cells, colon cancer cells, CNS cancer cells, head-and-neck cancer cells, etc. The sample used may be any isolated pathologic cell, or a tissue, organ, tissue sample, body fluid, etc. In a specific embodiment, the method is carried out on a biopsy from a subject, typically a biopsy section. The method may be performed on a sample collected from a subject and stored, optionally in frozen state, or extemporaneously, upon collection from the subject.

Determination of responsiveness or sensitivity to $\gamma\delta$ T cell comprises, within the context of the invention, an assessment of cell surface expression of an ATP synthase polypeptide or a fragment thereof. Indeed, the present invention now shows that ATP synthase participates in $\gamma\delta$ T cell activation, and is directly involved in TCR recognition and binding. The invention also shows that cells that are sensitive to $\gamma\delta$ T cells express at their surface an ATP

synthase, while such protein is normally expressed intracellularly (i.e., essentially in mitochondria). By detecting ATP synthase cell surface expression, sensitivity of a target cell to $\gamma\delta T$ cells may thus be evaluated.

5 Within the context of this invention, an ATP synthase polypeptide designates any polypeptide, protein or peptide fragment of an ATP synthase, as well as variants, isoforms and orthologs thereof. The sequence of ATP synthase genes and proteins has been disclosed in the prior art literature, and is available in various gene libraries. In particular, the amino acid sequence of a human ATP synthase protein is available on SwissProt Data base under
10 the following accession numbers : F1- α subunit: P25705; F1- β subunit: PO6576; F1- γ subunit: P36542; F1- δ subunit: 30049; F1- ϵ subunit : P56381. The nucleotide sequence of a human ATP synthase cDNA is available on GenBank Data base under the following accession numbers : F1- α subunit: BT007209; F1- β subunit: NM01686; F1- γ subunit: XM292254; F1- δ subunit: NM001687; F1- ϵ subunit : BT007293. Further information
15 regarding the cloning, sequence, characteristics, expression and structure-function of a human ATP synthase gene or protein can be found in Kataoka,H. and Biswas,C., *Biochim. Biophys. Acta* 1089 (3), 393-395 (1991) ; Neckelmann,N., et al., *Genomics* 5 (4), 829-843 (1989) ; Matsuda,C., et al., *J. Biol. Chem.* 268 (33), 24950-24958 (1993) and *Biochim. Biophys. Acta* 1130 (1), 123-126 (1992) or Tu,Q., et al., *Biochem. J.* 347 Pt 1, 17-21
20 (2000), incorporated therein by reference.

The mitochondrial ATP synthase is typically composed of an intramembrane sector termed F₀ and an extramembrane sector termed F₁. The F₁ sector comprises α , β , γ , δ and ϵ subunits in an $\alpha_3\beta_3\gamma\delta\epsilon$ polypeptidic complex. At least the α and β polypeptides show
25 ectopic expression and can be found at the surface of pathologic cells, such as tumor cells.

Accordingly, in a specific embodiment, the ATP synthase polypeptide is an ATP synthase α subunit.

In an other specific embodiment, the ATP synthase polypeptide is an ATP synthase β
30 subunit.

In a further specific embodiment, the ATP synthase polypeptide is a complex formed between an ATP synthase α and β subunits.

In an other specific embodiment, the method comprises detecting a portion of an ATP synthase, typically an extracellular domain of an ATP synthase α or β subunit.

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Determination of said cell surface expression can be performed using a variety of techniques known per se, such as by ligand binding, immunohistochemistry, etc. In a specific embodiment, the method comprises incubating the biological sample comprising said test, target or pathogenic cells (e.g., cultured cells, tissue or organ) with a ligand
40 specific for an ATP synthase polypeptide or a portion thereof, and assessing binding of said

ligand to said cell, tissue or organ. In a more preferred embodiment, the ligand is an antibody or a fragment or derivative thereof (e.g., a Fab or Fab'2 fragment, a CDR region, a SCFV, etc.). The ligand or antibody may be labelled, e.g., by fluorescent, enzymatic, luminescent, radioisotope, etc. labels, to facilitate binding determination.

5

The ATP synthase polypeptide, particularly the extracellular domains thereof, may be detected by indirect immunofluorescence using commercially available antibodies. As a specific example, antibodies specific for the α or β subunits may be found at Molecular Probes, Inc., under the following references:

- 10 . anti ATP synthase subunit alpha : clone 7H10 (product A-21350)
- . anti ATP synthase subunit beta : clone 3D5 (product A-21351)

In a specific embodiment, the method comprises providing a biopsy from the patient and determining cell surface expression of an ATP synthase polypeptide or a portion thereof by
15 immunohistochemistry using an antibody.

As mentioned, the method allows the design of improved or customized therapies, by determining which patients would best respond to particular $\gamma\delta$ T cell-mediated therapies. In this regard, the $\gamma\delta$ T cell-mediated therapy may be a direct in vivo therapy (e.g., comprising
20 the injection of a drug that stimulates $\gamma\delta$ T cells in said patient) or an ex vivo cell therapy (e.g., comprising the injection of ex vivo activated $\gamma\delta$ T cells to said patient).

The invention also provides novel methods of stimulating $\gamma\delta$ T cells, comprising contacting $\gamma\delta$ T cells with an ATP synthase polypeptide or a TCR-binding fragment thereof.
25 Alternatively, the invention also provides methods of stimulating $\gamma\delta$ T cells comprising contacting $\gamma\delta$ T cells with an apolipoprotein A1 polypeptide or a variant or ATP synthase- or TCR-binding fragment thereof. The present invention indeed discloses that Apolipoprotein A-I is a ligand of ATP synthase and of the TCR of lymphocytes T γ 9 δ 2, so that this polypeptide or, more preferably, derivatives thereof, represent valuable therapeutic
30 agents to mediate $\gamma\delta$ T cell therapy. These derivatives are preferably designed to have an increased affinity for the cell surface-expressed ATP synthase and/or for the TCR γ 9 δ 2, allowing the specific targeting of this lymphocyte population toward pathologic (e.g., tumor) cells.

35 These stimulation methods can be carried out in vivo, in vitro or ex vivo.

In a specific embodiment, the method comprises contacting $\gamma\delta$ T cells with a complex comprising an ATP synthase polypeptide or a TCR-binding fragment thereof linked to an apolipoprotein A1 polypeptide. In a further preferred embodiment, the ATP synthase

polypeptide or fragment is immobilized on a support. Indeed, as shown in the examples, solid support coated with ATP synthase polypeptide is able to stimulate $\gamma\delta$ T cells. The support may be a bead, column, plate, etc. The $\gamma\delta$ T cells are preferably human cells.

- 5 In this respect, an object of this invention resides in a product comprising, immobilized on a support, an ATP synthase polypeptide or a TCR-binding fragment thereof.

A further object of this invention resides in a method of increasing sensitivity of a target cell to $\gamma\delta$ T cell lysis or cytotoxicity, comprising causing or increasing cell surface
10 expression of an ATP synthase polypeptide or a portion thereof in said target cell.

A further object of this invention is a method of screening, selecting or identifying a compound, comprising:

- 15 a) separately contacting $\gamma\delta$ T cells with an ATP synthase polypeptide or a TCR-binding fragment thereof in the presence and in the absence of a candidate compound and,
b) screening, selecting or identifying a compound that modulates activation of said $\gamma\delta$ T cells by said ATP synthase polypeptide or a TCR-binding fragment thereof.

The method is typically conducted in vitro, in any suitable device, such as a plate, tube,
20 flask, pouch, etc. The method is particularly suited for use in multi-well plates, to screen several compounds in parallel. In the above screening methods, the ATP synthase polypeptide or TCR-binding fragment thereof may be in suspension or immobilized on a support, such as a bead or at the surface of the device itself. Also, the method may be conducted in the presence of an apolipoprotein A1 polypeptide. Furthermore, the method is
25 suitable for selecting or designing or improving apolipoprotein A1 polypeptide variants that modulate activation of $\gamma\delta$ T cells. The activity of $\gamma\delta$ T cells may be assessed using a number of biological assays, such as proliferation, target cell lysis, cytokine release, etc. The method is particularly suited to identify compounds that activate $\gamma\delta$ T cells.

- 30 Further aspects and advantages of this invention will be disclosed in the following experimental section, which should be regarded as illustrative and not limiting the scope of this application.

MATERIAL AND METHODS

35

1) Tumor cell lines, T cell clones and cultures

Daudi, Raji, RPMI 8226, K562, Jurkat, Molt-4, and U937, SK-NP, G401, G402, and 786.0 were obtained from ATCC. Awells (EBV⁺ lymphoblastoid B cell line) is from the International Histocompatibility Workshop (IHW#9090). C1R (HLA-A*B*-LCL) and RPMI

8866 (B-LCL) were obtained from Drs P.Lebouteiller and M.Colonna respectively. All tumor cell lines were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum (Invitrogen) except for serum deprivation experiments. In this case, cells were washed once in RPMI 1640, incubated for 1 hour at 37°C in serum free culture medium (hybridoma sfm, Invitrogen), pelleted and reincubated for at least 16 hours at 37°C in sfm before use. The G25 (V γ 9V δ 2) and 73R9 (V γ 8V δ 3) clones were obtained as described for G42 and G115 (Allison et al., 2001; Davodeau et al., 1993) by anti-V δ monoclonal antibody selection and subsequent amplification using PHA and IL2 and cloning.

10 3) Fluorescence analysis and antibodies

Immunofluorescence stainings were performed in PBS containing 1%BSA and devoid of serum using FITC-conjugated goat F(ab) $'_2$ anti-mouse Ig antibody (Caltag) as the second step reagent. Irrelevant isotype-matched control antibodies were used as negative controls. In apolipoprotein binding experiments, serum-deprived cells were incubated with serum or purified protein preparations in PBS containing 1%BSA, at room temperature, 30 minutes prior to antibody staining. 7H10 (anti- α -ATP synthase) and 3D5 (anti- β -ATP synthase) and 7F9 (anti- γ -ATP synthase) are from Molecular Probes. 4H1 (anti-human apoA-I) (Collet et al., 1997) was obtained from Dr Y. Marcel (Ottawa).

20 4) Generation of M5A12D10 hybridoma and antibody purification

Balb/c mice were injected intraperitoneally four times at two-weeks intervals with 15x10⁶ Daudi cells washed and resuspended in PBS. Hybridoma were obtained by fusion of spleen cells with P3X63Ag8 myeloma cells and were selected on the basis of tumor cell staining. Subcloned hybridoma were subsequently amplified in sfm medium and antibodies were purified on protein G affinity columns (Pharmacia), neutralized, dialyzed against PBS and concentrated (Harlow and Lane, 1988). Finally, antibodies were tested for their ability to modulate lysis of Daudi cells by $\gamma\delta$ effectors, leading to the selection of the M5A12D10 antibody (IgG1).

30 5) Purified proteins

The human and bovine ligands of M5A12D10 (hM5L and bM5L respectively) were isolated by affinity chromatography: human and fetal calf serum diluted 1/20 in 3 M NaCl and 50 mM Tris pH 7 were passed through the column carrying the covalently attached antibody. After washing (last wash was in 3 M NaCl, 10 mM Tris pH 7), bound proteins were eluted with 100 mM glycine pH 2.7. Isolation of ApoA-I from High Density Lipoproteins (HDL apoA-I) by ion-exchange chromatography has been already described (Mezdour et al., 1987). Purity of apoA-I was checked by Western blot analyses using different antibodies directed against human apoB, apoA-II, apo-C and apoA-I. The apolipoprotein A-I homogeneity was more than 99% (as measured by densitometry after

SDS-PAGE and silver staining). Purified bovine ATP synthase (F1 subunit) (Lutter et al., 1993) was obtained from John E. Walker (Cambridge, UK).

6) Production of soluble fluorescent tetrameric TCR and usage for cell staining

5 The G115 (V γ 9V δ 2) (3) and 73R9 (V γ 8V δ 3) extracellular γ and δ chains (the latter carrying a short 3' Biotin tag) were expressed in *Escherichia coli*, then refolded together by rapid dilution in 1 L of 1 M L-arginine, 0.1 M Tris-HCl, pH 8.0 and 0.2 mM reduced/0.2 mM oxidized glutathione. After dialysis against 10 mM Tris-HCl pH 8.0, concentration by cation exchange chromatography at pH 5.5, and purification by size
10 exclusion chromatography at pH 8.0, the refolded protein was biotinylated for 4 h at 30°C with 6 μ g/ml BirA and excess of free biotin was removed by dialysis against 10 mM Tris-HCl pH 8.0, 150 mM NaCl. Tetramers of $\gamma\delta$ TCR heterodimers were obtained by mixing the biotinylated TCR with phycoerythrin-labeled streptavidin (Biosource) at a molar ratio of 10:1. For staining, $2 \cdot 10^5$ cells were incubated at room temperature for 45 min with
15 phycoerythrin-labeled $\gamma\delta$ TCR tetramers at a concentration of TCR of 30 μ g/ml in PBS plus 1% BSA. Background staining was determined using phycoerythrin-labeled streptavidin at the same concentration.

7) Protein identification by proteomic analysis and mass spectrometry

20 Immunopurified material was analyzed using one dimensional electrophoresis and visualized by coomassie blue staining. Protein bands were excised from the gel and subjected to several washing steps, reduction alkylation reaction, in-gel trypsin digestion with modified trypsin (Promega, Madison, WI) at 25 ng/ μ l in 50 mM NH₄HCO₃ and finally followed by peptide extraction. The peptides purified with ZipTip C18 (Millipore)
25 were mixed with equal volumes (0.5 ml) of a saturated α -cyano-4-hydroxycinnamic acid in 50% acetonitrile, 0.1% TFA onto the MALDI target and allowed to air-dry. Peptide mass fingerprinting were obtained by using a PE Biosystems MALDI-TOF mass spectrometer (Voyager DE STR, Foster City, CA, USA) on each protein band. Unknown proteins were identified using the data base fitting program MS-Fit (Protein Prospector,
30 (<http://prospector.ucsf.edu>)), searching against all eukaryotic entries in Swiss Prot and NCBI non redundant protein data bases. We considered the identification positive when a minimum of four measured peptide masses were matched and provided at least around 20% sequence coverage. Mass accuracy of 10 ppm was obtained with internal calibration using auto-digestion peaks of trypsin (M+H⁺, 842.51, 2211.10, and 2283.18).

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8) Chromium and cytokine release assays

2h-⁵¹Cr-release assays were performed in standard conditions except for the use of serum-free conditions in some experiments: in sensitization experiments with apolipoprotein preparations, target tumor cells were serum-deprived as described for facs analysis, loaded

with ^{51}Cr (100 $\mu\text{Ci}/10^6$ cells, 1 hour, 37°C), extensively washed in RPMI and resuspended in sfm medium. Effector T cells cultivated in serum-containing medium were washed extensively in RPMI, incubated for 2 hours in sfm at 37°C and resuspended in sfm medium. Target cells (3000/well, in triplicates) were first incubated with apolipoprotein preparations or serum-containing medium for 30 min at room temperature in 96-well round-bottom microculture plates at room temperature prior to the addition of effector cells. Cells were then pelleted and incubated at 37°C for 2 hours. Supernatants were recovered for ^{51}Cr release measurement. Spontaneous release (in the absence of effectors) was subtracted from experimental data and was in the 10-30% range of maximum release (effector cells replaced by same volume of 0.1 M HCl). Specific lysis was calculated as the percentage of maximum release. For cytokine release measurements, similar experiments were performed. supernatants were harvested after 4 hours (TNF α) or 20 hours (IFN γ). IFN γ was titrated by a specific Elisa technique, whereas TNF α concentration was assessed by a biological assay based on WEHI cells viability.

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9) Binding assays

The binding experiments were performed at 4°C for 2 hours as previously described (Barbaras et al., 1994). Briefly, cells (9 μg of cell proteins per point) were incubated in PBS for 2 hours at 4°C with increasing concentrations of labeled apoA-I. Cells were filtered on 0.22 μm filters (GVWP Millipore-France) and washed four times with 1% BSA in PBS. Filters were used for radioactivity measurements. Non-specific binding was determined in the presence of a 100-fold excess (as compared to the K_D value) of the corresponding unlabeled ligand. Binding was analyzed using a weighted non-linear curve-fitting program, based on the LIGAND analysis program (Prism-GraphPad).

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10) Immobilization of proteins on latex beads.

10^7 sulfate latex beads (Interfacial Dynamics corp., Portland, OR) were washed in PBS and incubated with apolipoproteins (100 $\mu\text{g}/\text{ml}$), F1-ATP synthase (0.4 mg/ml) or a mixture of both under constant agitation at room temperature for 16 hours. Beads were then washed, saturated for 3 hours in PBS containing 1% BSA and washed extensively in PBS before use. Control "empty" beads were similarly saturated with BSA. In stimulation experiments, beads were mixed with cells at a 1:1 ratio.

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11) Immobilization of proteins for SPR (Biacore) analysis.

ApoA-I and ATP synthase (F1 subunit) were immobilized by amine linkage on CM5 chips (Biacore AB) following NHS-EDC activation; V γ 9V δ 2-biotinylated soluble TCRs were immobilized on SA streptavidin-coated chips (Biacore AB) and binding was analyzed in a Biacore 3000 apparatus (Biacore AB). Soluble ligands were injected at a flow rate of 20 $\mu\text{l}/\text{min}$, exposed to the surface for 240 s (association phase) followed by a 240-s flow

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running during which the dissociation occurred. Sensorgrams are representative of specific interactions (differential response) where non-specific binding that occurred on flow cell 1 was deduced from binding that occurred on flow cell 2. Results are expressed as resonance units (RU) as a function of time in seconds.

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RESULTS

In an attempt to characterize such antigens, we raised murine monoclonal antibodies against the Burkitt's lymphoma Daudi, a cell line which is lysed by a large majority of V γ 9V δ 2 T cell clones (Davodeau et al., 1993; De Libero et al., 1991). We selected monoclonal antibodies (mAb) for their differential binding to Daudi and Raji (a non-activating Burkitt's lymphoma) and for their ability to interfere with V γ 9V δ 2 T cell recognition of Daudi cells. One mAb (#M5A12D10, hereafter referred to as M5) which fulfilled both criteria was selected for further studies. M5 mAb stained Daudi cells as well as several other hematopoietic tumors reported to be recognized by V γ 9V δ 2 T cells, including cell lines of lymphoid (MOLT-4, RPMI 8226) and myeloid (K562, U937) origins. By contrast several tumor cells resistant to V γ 9V δ 2 T cell killing such as Raji and B lymphoblastoid cells were not stained by M5 mAb (Fig.1a). As M5 mAb binding to tumor cells was dependent on the presence of serum in cell culture medium (Fig.1b), M5 was subsequently used to immunopurify a putative ligand from bovine serum. A protein (M5L) running in polyacrylamide gels as a ~28kDa polypeptide could be isolated and mass spectrometry analysis of tryptic peptide digests identified apolipoprotein A-I as the likely ligand. M5 immunopurified a similar protein from human serum (hM5L) and its identity to apoA-I was confirmed by its reactivity in immunoblotting experiments using the anti-human apoA-I monoclonal antibody 4H1 (Fig.1d). Accordingly, like M5 Ab, 4H1 stained Daudi and RPMI 8226, another tumor line frequently killed by V γ 9V δ 2 T cells, but not Raji cells following their incubation with a human lipid-free form of human apoA-I prepared from high density lipoprotein particles (HDL-apoA-I) by ion exchange chromatography (Fig.1c). In order to assess the putative involvement of apo A-I in V γ 9V δ 2 cytolytic activity, we performed cytotoxicity assays in serum-free medium (sfm), using target cells depleted of serum after an overnight culture in sfm (Fig.2). Cytolytic activity of V γ 9V δ 2 T cell clones against Daudi cells was decreased in serum-free conditions, and restored by addition of serum during the cytotoxicity assay. Similarly, addition of purified M5L increased V γ 9V δ 2 T cell-mediated lysis in a dose-dependent manner. By contrast Daudi cell lysis by a control V γ 8V δ 3 T cell clone (#73R9) was neither affected by serum deprivation nor by addition of M5L (Fig.2). A similar effect on V γ 9V δ 2 T cell cytotoxicity was observed using HDL-apoA-I or a commercial apoA-I preparation (Sigma, data not shown). Thus, tumor lysis by V γ 9V δ 2 T cells is specifically affected by soluble extracellular apoA-I. Modulation of V γ 9V δ 2 T cell activity by apo A-I could be due to apoA-I recognition by either the V γ 9V δ 2 TCR itself or by accessory receptors such as toll-like receptors which are

known to be expressed on various conventional and non-conventional T cell subsets (Caramalho et al., 2003 ; Mokuno et al., 2000 ; Sakaguchi, 2003). Possible involvement of apoA-I in TCR engagement was studied by using recombinant soluble forms of the V γ 9V δ 2 TCR derived from the G115 clone (Allison et al., 2001), which were biotinylated and tetramerized by fluorescent streptavidin (hereafter referred to as TCR tetramers). Like M5 mAb, V γ 9V δ 2 TCR tetramers bound to the V γ 9V δ 2 target cells Daudi, K562 and more weakly to RPMI 8226, but neither to Raji cells nor to B lymphoblastoid cell lines (Fig.3a and data not shown). As a control, V γ 8V δ 3 TCR tetramers derived from clone 73R9 bound to Daudi but to a much lesser extent to K562 cells (Fig.3a) indicating that these two TCRs target distinct structures on the tumor cell surface. K562 cells, which yielded the brightest staining levels with V γ 9V δ 2 TCR tetramers, were used in further experiments. V γ 9V δ 2 TCR tetramer binding to K562 was decreased when cells were cultured for 18h in the absence of serum (not shown) and increased following addition of serum or human apoA-I (hM5L or HDL-apoA-I). Importantly apoA-II, whose hydrophobicity was similar to that of apoA-I, did not enhance V γ 9V δ 2-TCR tetramer binding (Fig.3b). This suggested that the effect of apoA-I was not merely due to a non-specific adsorption of TCR tetramers, which might have been induced by hydrophobic compounds. Accordingly neither serum, nor apoA-I, affected the binding of V γ 8V δ 3 TCR tetramers (Fig.3b). Also consistent with a specific effect of apoA-I on V γ 9V δ 2 TCR tetramer binding, pre-incubation of cells with M5 and 4H1 antibodies decreased TCR tetramer binding to cells coated with hM5L. 4H1 decreased tetramer binding on cells preincubated with HDL-derived apoA-I whereas M5 did not (Fig.3c). This goes along with the observation that M5 mAb does not bind to HDL-derived apoA-I (not shown) and suggests structural differences between the immunopurified and the HDL-derived forms of apoA-I, although both are recognized by 4H1 and promote TCR binding. Although soluble V γ 9 δ 2 TCR no longer bound to serum-deprived Daudi cells (data not shown), they still stained serum-deprived K562 cells (Fig.3b). This could be explained by the persistence of residual apolipoproteins on the cell surface. However, it remained possible that the soluble TCR bound to an as yet unidentified structure and that this binding was increased by apoA-I.

We have recently shown that components of the mitochondrial enzyme ATP-synthase (AS) can be expressed on the surface of hepatocytes, and that this enzyme constitutes a high affinity receptor for free, delipidated apoA-I on these cells (Martinez et al., 2003). We studied expression of components of this enzyme on the surface of V γ 9V δ 2 tumor targets. Daudi, K562 and RPMI 8226 were stained by mAb against the α chain of AS (α AS) whereas Raji, leukaemic T cells and B-LCL were not (Fig.4a). Four kidney tumors which were lysed by V γ 9V δ 2 CTL also expressed an α AS-related surface component (Fig.4c). The β subunit of AS was also detected on Daudi, K562 and U937, a monocytic line not consistently killed by V γ 9V δ 2 T cells. This chain was undetectable on RPMI 8226 and the kidney tumors (Fig.4b,c). Therefore tumor susceptibility to V γ 9V δ 2 lysis more strongly

correlated with expression of the α AS subunit. Scatchard analysis of the binding of iodinated apoA-I to Daudi cells revealed a single binding receptor on these cells ($K_D = 0.8 \times 10^{-7} \text{M}$; Fig.1e). Since Daudi cells did not express other putative apoA-I receptors, such as Scavenger Receptor B1 (Murao et al., 1997) or ABCA-1 (Chambenoit et al., 2001) (data not shown), these experiments strongly suggested that AS was the apoA-I receptor on tumor cells.

Two sets of experiments were performed to assess the involvement of AS in tumor cell recognition by V γ 9V δ 2 T cells. Firstly, antibodies to apoA-I and to the extramembrane (F1) subunit of AS were tested for their ability to modulate lymphokine secretion by V γ 9V δ 2 T cells following incubation with Daudi cells. IFN γ secretion was strongly inhibited by anti- α AS, anti- β AS and M5, as compared to a control Ig. This inhibition was not merely due to Ab toxicity since V γ 9V δ 2 T activation was largely restored by addition of isopentenyl pyrophosphate (IPP), a previously described V γ 9V δ 2 T cell antigen (Fig.5).

Secondly, the involvement of AS in V γ 9V δ 2 T cell activation was studied by testing the ability of mitochondrial bovine AS immobilized on latex beads with and without apoA-I to stimulate V γ 9V δ 2 T cells. Beads carrying apoA-I only or none of these proteins were also tested (Fig.6). Co-culture with AS-coated beads induced a strong TNF α secretion by V γ 9V δ 2 clones, whereas V γ 8V δ 3 and $\alpha\beta$ clones, otherwise able to produce TNF α after stimulation by phorbol myristate acetate and calcium ionophore, were not activated (Fig.6a). We could not demonstrate any stimulatory activity of immobilized apoA-I alone. Similar experiments performed in serum-free conditions indicated a stimulatory activity of beads carrying AS alone, and a relatively minor increase of this stimulatory activity following addition of soluble apoA-I (Fig.6b). This would suggest an accessory role of apoA-I in the stimulatory activity of AS. However a possible carryover of apoA-I or related apolipoproteins by effector cells cannot be ruled out. When added onto fresh PBL, beads carrying AS and apoA-I induced selective TNF α production by a large fraction of V δ 2 $^+$ T cells but had no significant effect on the $\alpha\beta$ and NK cell populations (Fig.6c).

Altogether the above results suggested a highly specific and TCR-mediated activation of V γ 9V δ 2 cells by apoA-I and AS. However, the respective contribution of AS and apoA-I in $\gamma\delta$ T cell / tumor cell interactions remained unclear. Although anti-apoA-I antibodies had a substantial inhibitory effect on cytotoxicity and lymphokine secretion, these effects as well as the modulatory properties of apoA-I could be explained by the proximity of apoA-I and AS on the cell surface, possibly generating indirect perturbation of TCR-AS interactions. Surface plasmon resonance analysis (SPR) has been previously used to demonstrate interactions between apoA-I and ATP synthase (Martinez et al., 2003). We followed a similar approach to assess TCR/apoA-I/AS interactions, after immobilization of either apoA-I, soluble G115 TCR or purified AS onto SPR chips. Immobilized apoA-I specifically interacted with soluble monomeric G115 TCR ($K_D \sim 0.8 \mu\text{M}$) whereas interaction with the 73R9 TCR was too low to be measured (Fig.7a,b). Conversely,

immobilized G115 TCR specifically interacted with soluble apoA-I, but not with apoA-II (Fig.7c). When soluble TCR were similarly tested on immobilized AS, a clear interaction was demonstrated for the V γ 9V δ 2 TCR ($K_D=1.5\mu M$) whereas the binding of the V γ 8V δ 3 TCR was low and could not be measured (Fig.7d,e). Thus SPR analysis confirmed the occurrence of specific interactions between V γ 8V δ 2 TCR, AS and apo A-I.

DISCUSSION

Our results strongly suggest that during interaction between V γ 9V δ 2 T cells and their tumoral targets, a ternary complex forms between the TCR on the T cell side and apoA-I plus AS on the tumor cell side. It is not clear whether the structure expressed on the surface of tumors is identical to mitochondrial AS : reactivity with available monoclonal antibodies suggests that the α AS and β AS chains can be independently expressed on various tumors, indicating some form of surface expression polymorphism of these proteins. Using a limited panel of tumor lines we found that sensitivity to V γ 9V δ 2 T cells correlated better with α AS than with β AS expression. Although AS might not be the primary tumor target antigen of V γ 9V δ 2 T cells but instead a cross-reactive unrelated entity, the stimulatory potential of immobilized mitochondrial AS would indicate that its homology to the putative primary antigen(s) is high enough to induce a potent V γ 9V δ 2 T cell stimulation.

Direct binding of apoA-I to AS (Martinez et al., 2003) and the TCR (our present data) have now been demonstrated. These interactions seem to be required for optimal T cell activation as V γ 9V δ 2 T cell activation and soluble TCR binding were markedly decreased by serum depletion and were restored by addition of exogenous soluble apoA-I. However, our present results suggest a hierarchical importance of these interactions for V γ 9V δ 2 T cell activation. Indeed, we could not demonstrate any stimulatory activity for immobilized apoA-I whereas AS appeared to be stimulatory by itself when immobilized on latex beads. SPR experiments however strongly suggest a direct interaction of apoA-I with both the TCR and AS. Thus a possible role for apoA-I could be the stabilisation of the interaction between the TCR and the AS, explaining the stimulatory effect of apoA-I in cytotoxicity assays.

ApoA-I is unlikely to be immunogenic by itself and, as already mentionned, immobilized apoA-I did not activate V γ 9V δ 2 T cells. However, apoA-I may serve as a carrier for antigenic ligands such as phosphoantigens, either derived from the tumor cells themselves or from the extracellular medium, thus permitting their "presentation" by a surface AS-related structure. This possibility would be consistent with the recently described correlation between endogenous production of mevalonate pathway metabolites (including IPP) by tumor cells and their susceptibility to V γ 9V δ 2 T cell-mediated lysis (Gober et al., 2003).

Ectopic expression of components of AS is not unprecedented as the presence of AS was previously described on the surface of K562 cells (Das et al., 1994) and endothelial cells

- (Moser et al., 1999) and linked to immunomodulatory effects in one case. The α/β AS subunits are also found on hepatocytes where they promote binding of free apoA-I and display enzymatic activity (Martinez et al., 2003). Whether these components are also involved in an enzymatic complex on tumor cells is not known yet. Although expression of this apoA-I receptor on normal tissue cells could potentially induce V γ 9V δ 2 T lymphocyte-mediated autoimmunity, several recent studies have described mechanisms involving NK-like receptors and permitting efficient control of this potential self reactivity (Fisch et al., 1997; Halary et al., 1997).
- Besides, together with the high expression of LDL receptors and apoE in intraepithelial $\gamma\delta$ lymphocytes (Fahrer et al., 2001), and with studies documenting apoE binding to ATP-synthase (Mahley et al., 1989), these data open a new field of investigations linking lipid metabolism and anti-tumor immunosurveillance.

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CLAIMS

1. A method of assessing the sensitivity of a pathologic cell to $\gamma\delta$ T cell lysis or cytotoxicity, comprising determining in vitro or ex vivo whether said pathologic cell expresses, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being an indication of the sensitivity of the pathologic cell to $\gamma\delta$ T cell lysis or cytotoxicity.
2. A method of assessing whether a cell is a target cell for $\gamma\delta$ T cells, comprising determining in vitro or ex vivo whether said cell expresses, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being an indication that the cell is a target of $\gamma\delta$ T cells.
3. A method of assessing the responsiveness of a patient to $\gamma\delta$ T cell-mediated therapy, comprising determining whether said patient contains pathologic cells, tissues or organs that express, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being an indication of the responsiveness of the patient to $\gamma\delta$ T cell-mediated therapy.
4. A method of selecting patients for a $\gamma\delta$ T cell-mediated therapy program, comprising determining whether a patient contains pathologic cells, tissues or organs that express, at the surface thereof, an ATP synthase polypeptide or a portion thereof, the cell surface expression of an ATP synthase polypeptide or a portion thereof being a criteria for selecting said patient for said $\gamma\delta$ T cell-mediated therapy program.
5. The method of claim 1, 2, 3 or 4, wherein said ATP synthase polypeptide is an ATP synthase α subunit.
6. The method of claim 1, 2, 3 or 4, wherein said ATP synthase polypeptide is an ATP synthase β subunit.
7. The method of claim 1, 2, 3 or 4, wherein said portion is an extracellular domain of an ATP synthase α or β subunit.
8. The method of claim 1, 2, 3 or 4, wherein said determination comprises incubating said cell, tissue or organ with a ligand specific for an ATP synthase polypeptide or a portion thereof and assessing binding of said ligand to said cell, tissue or organ.

9. The method of claim 8, wherein said ligand is an antibody or a fragment or derivative thereof.
10. The method of claim 3 or 4, comprising providing a biopsy from said patient and
5 determining cell surface expression of an ATP synthase polypeptide or a portion thereof by immunohistochemistry.
11. The method of claim 1, wherein said pathologic cell is a tumor cell, a pathogen-infected cell, or a pathologic immune cell.
- 10 12. The method of claim 3 or 4, wherein said patient is a human subject.
13. The method of claim 3 or 4, wherein the $\gamma\delta$ T cell-mediated therapy comprises the injection of a drug that stimulates $\gamma\delta$ T cells in said patient.
- 15 14. The method of claim 3 or 4, wherein the $\gamma\delta$ T cell-mediated therapy comprises the injection of ex vivo activated $\gamma\delta$ T cells to said patient.
- 15 15. A method of stimulating $\gamma\delta$ T cells, comprising contacting $\gamma\delta$ T cells with an ATP
20 synthase polypeptide or a TCR-binding fragment thereof.
16. A method of stimulating $\gamma\delta$ T cells, comprising contacting $\gamma\delta$ T cells with an apolipoprotein A1 polypeptide or a variant or ATP synthase- or TCR-binding fragment thereof.
- 25 17. The method of claim 15 or 16, comprising contacting $\gamma\delta$ T cells with a complex comprising an ATP synthase polypeptide or a TCR-binding fragment thereof linked to an apolipoprotein A1 polypeptide.
- 30 18. The method of claim 15 or 17, wherein said ATP synthase polypeptide or fragment is immobilized on a support.
19. The method of claim 15 or 16, wherein said $\gamma\delta$ T cells are human cells.
- 35 20. A method of increasing sensitivity of a target cell to $\gamma\delta$ T cell lysis or cytotoxicity, comprising causing or increasing cell surface expression of an ATP synthase polypeptide or a portion thereof in said target cell.
21. A method of screening, selecting or identifying a compound, comprising:

- a) separately contacting $\gamma\delta$ T cells with an ATP synthase polypeptide or a TCR-binding fragment thereof in the presence and in the absence of a candidate compound and,
- b) screening, selecting or identifying a compound that modulates activation of said $\gamma\delta$ T cells by said ATP synthase polypeptide or a TCR-binding fragment thereof.

Figure 1a

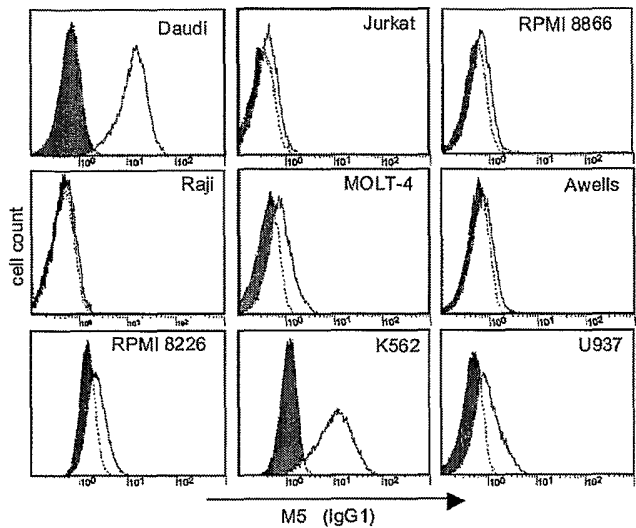


Figure 1b

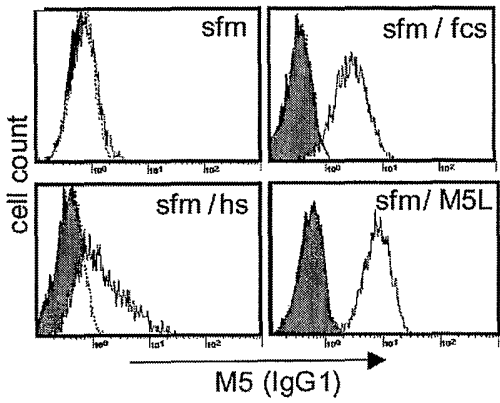


Figure 1c

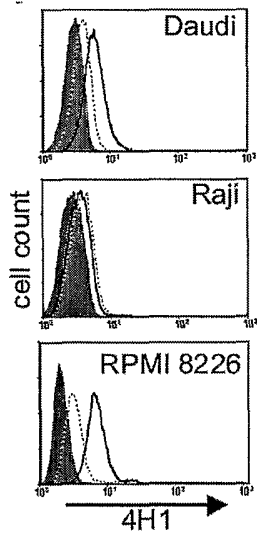


Figure 1d

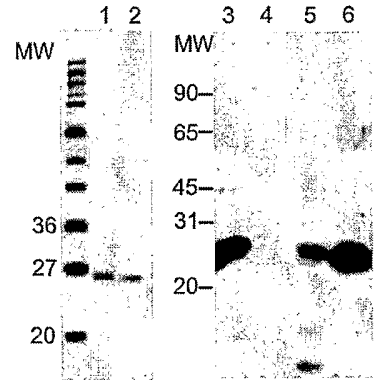


Figure 1e

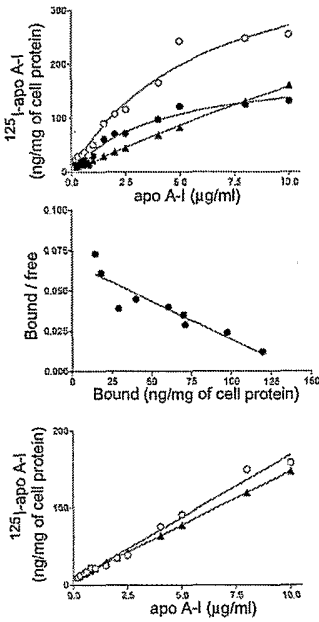


Figure 1

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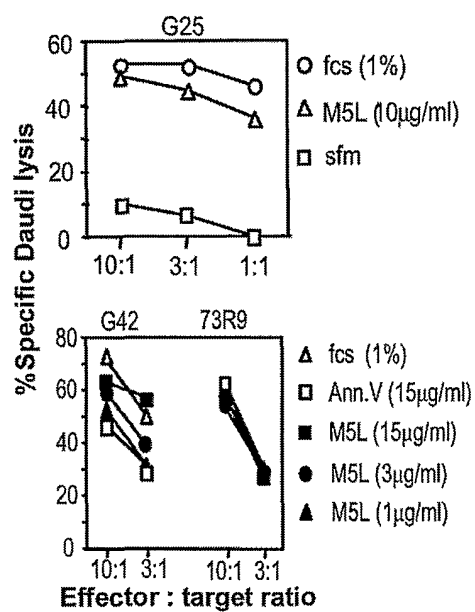
Figure 2

Figure 3

Figure 3a

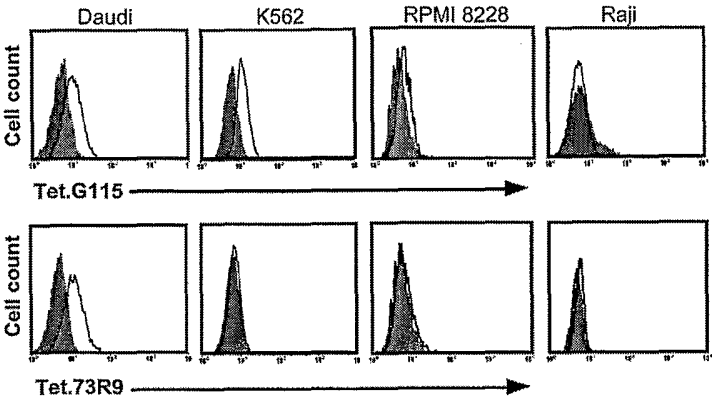


Figure 3b.

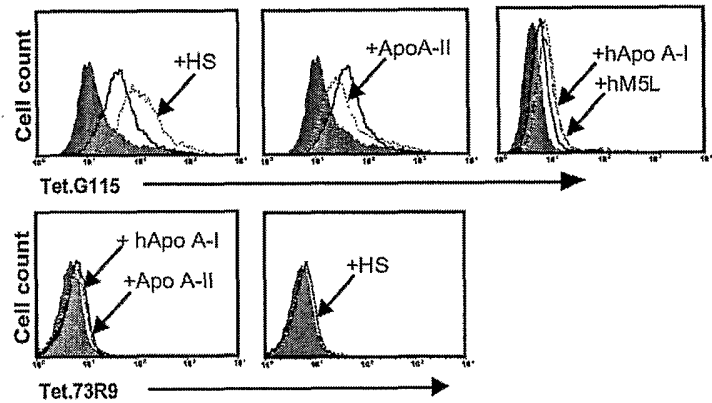


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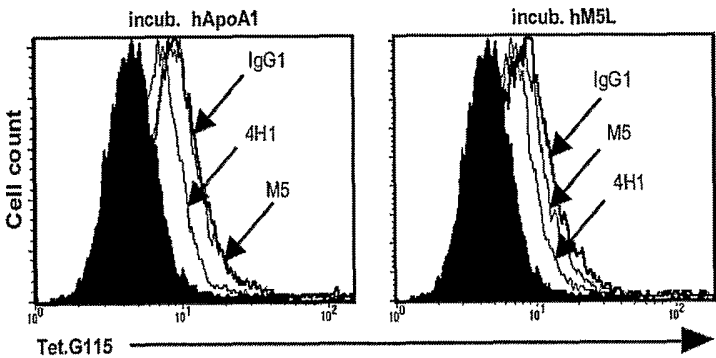


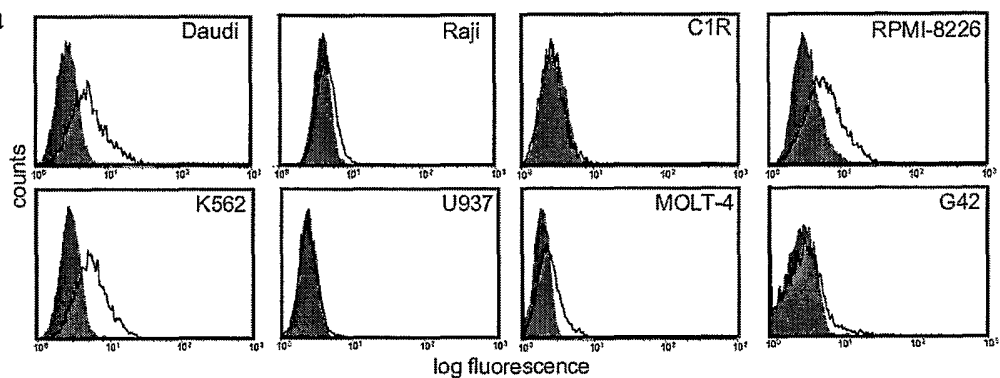
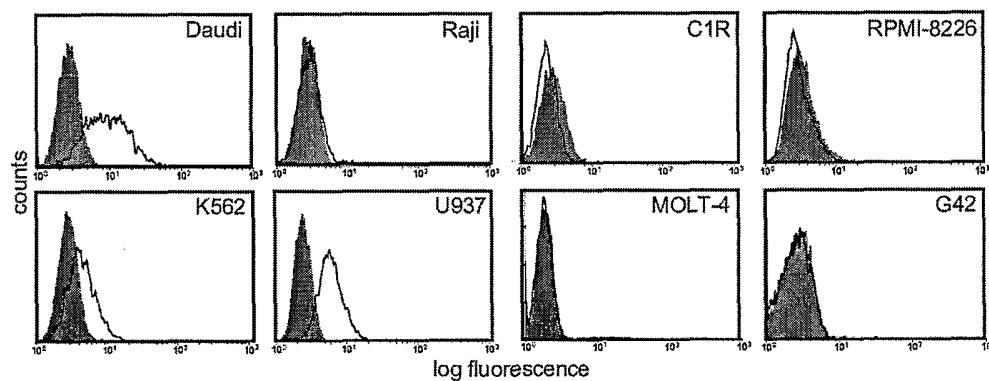
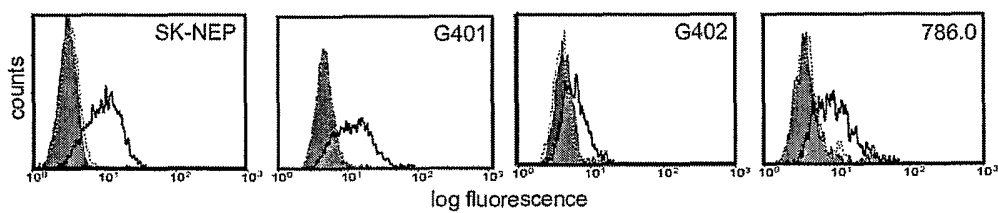
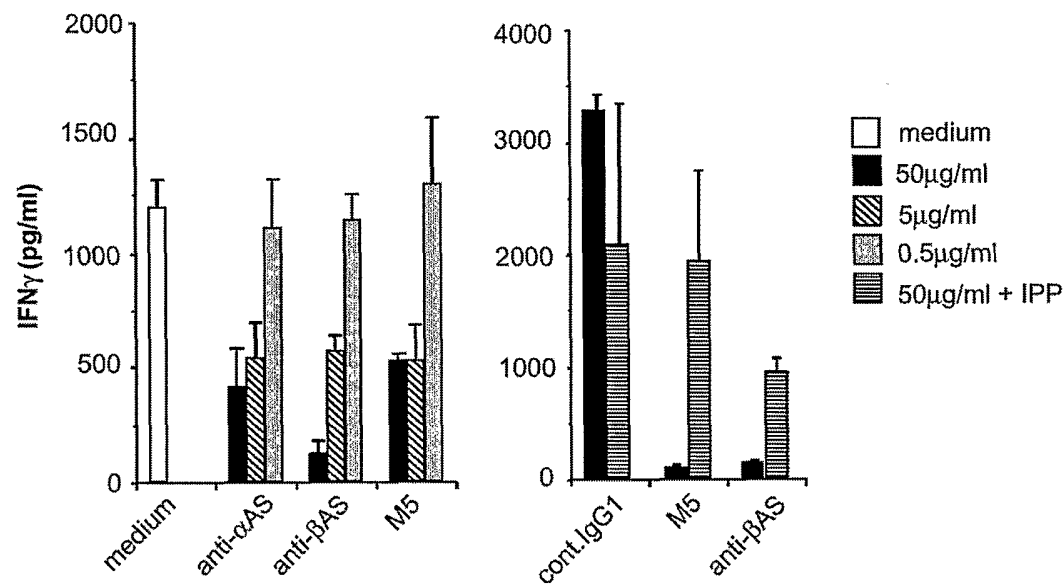
Figure 4**Figure 4a****Figure 4b****Figure 4c**

Figure 5



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Figure 6

Figure 6a

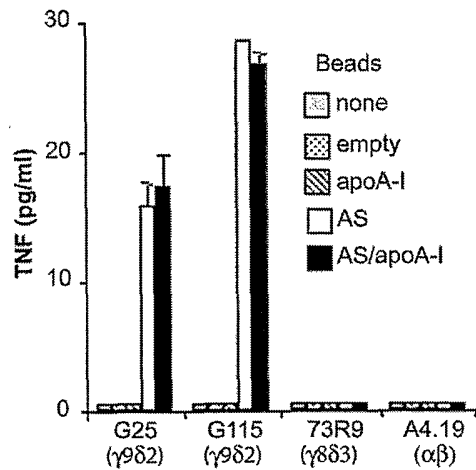


Figure 6b

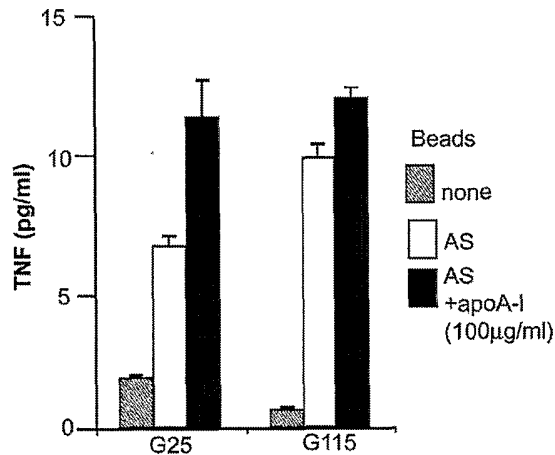


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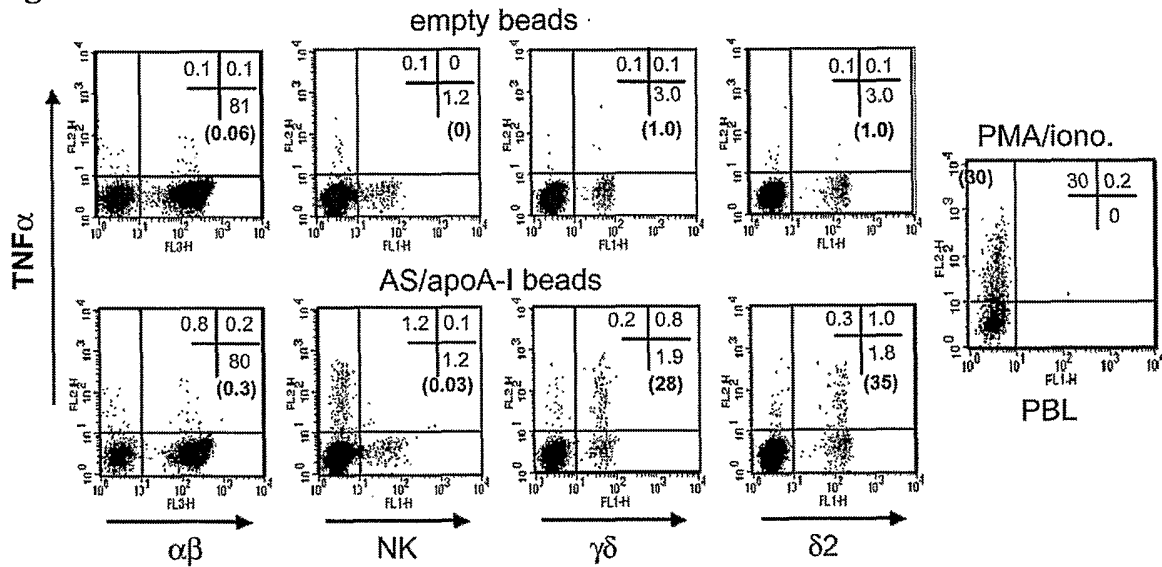


Figure 7

Figure 7a

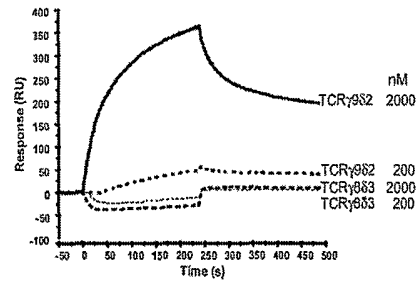


Figure 7b

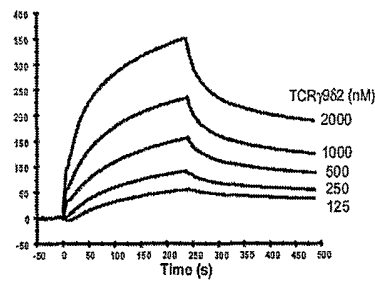


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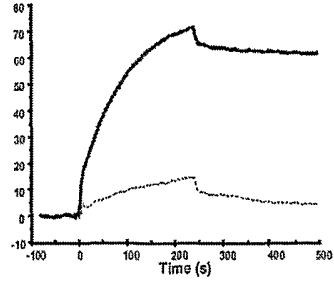


Figure 7d

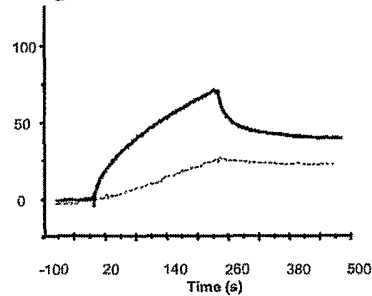


Figure 7e

