

Title: Immunoglobulins binding human V γ 9V δ 2 T cell receptors

5 Field of the invention: The present invention is in the field of medicine and relates to immunology. The invention relates to immunoglobulins binding T cells. In particular, the invention relates to immunoglobulins binding human V γ 9V δ 2 T cell receptors. The invention provides for immunoglobulin molecules that bind a human V γ 9V δ 2 T cell receptor, such as antibodies, single chain antibodies, or single domain antibodies, wherein the human V γ 9V δ 2
10 T cells may be modulated.

Background: The majority of $\gamma\delta$ peripheral blood lymphocytes (PBLs) in human adults express T-cell receptors (TCRs) comprising V γ 9 and V δ 2 regions. V γ 9V δ 2 T cells can react against a wide array of pathogens and tumour cells. This broad reactivity is understood to be
15 conferred by phosphoantigens which are able to specifically activate this T-cell subset in a TCR dependent fashion. The broad antimicrobial and anti-tumour reactivity of V γ 9V δ 2 T-cells suggest a direct involvement in immune control of cancers and infections. In addition to fighting disease, in some diseases or medical treatment V γ 9V δ 2 T cells may be overstimulated or inadvertently activated.

20 Hence, agents that can activate V γ 9V δ 2 T cells can be useful in the treatment of infections or cancer as these may promote V γ 9V δ 2 T cell reactivity towards the pathogen or infected cells or cancer. Furthermore, agents that block activation of V γ 9V δ 2 T cells may be useful in diseases or medical treatment where it is advantageous to reduce V γ 9V δ 2 T cell activation, i.e. wherein V γ 9V δ 2 T cells are overstimulated or inadvertently activated. Finally,
25 agents that can bind a V γ 9V δ 2 T cell, but do not have an effect on (phosphoantigen) activation of V γ 9V δ 2 T cells are useful for labelling cells, for example for selecting or identifying V γ 9V δ 2 T cells. Hence, there is a need in the art to provide for agents that can bind to V γ 9V δ 2 T cells, and for agents that can block phosphoantigen activation of V γ 9V δ 2 T cells or can activate V γ 9V δ 2 T cells.

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Summary of the invention: The current invention now provides for novel agents that can bind to V γ 9V δ 2 T cells. The agents provided are immunoglobulins. The immunoglobulins provided bind to a V γ 9V δ 2 T cell receptor. Surprisingly, it was found that the immunoglobulins provided by the current invention have a substantial sequence identity. Hence, in one aspect, the
35 present invention provides a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, comprising a CDR1 region, a CDR 2 region, and a CDR 3 region, wherein the CDR1 region comprises an amino acid sequence with at least 40 % sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG, and wherein the CDR2 region

comprises an amino acid sequence with at least 60 % sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG, and wherein the CDR3 region contributes to V γ 9V δ 2 T cell receptor binding; and wherein the immunoglobulin molecule is an immunoglobulin molecule that activates human V γ 9V δ 2 T cells.

- 5 Also disclosed are human V γ 9V δ 2 T cell receptor binding immunoglobulin molecules, comprising a CDR1 region and a CDR 2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40 % sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; and wherein the

CDR2 region comprises an amino acid sequence with at least 60 % sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; wherein preferably the immunoglobulin molecule is a single domain antibody.

Furthermore, such immunoglobulins comprise a CDR3 region, wherein the CDR3
5 region contributes to V γ 9V δ 2 T cell receptor binding and may have an effect on the action of the immunoglobulin molecule. This may, without being bound by theory, implicate the CDR3 sequence in the functionality of the immunoglobulin molecule, i.e. type of modulation such as blocking activation of V γ 9V δ 2 T cells, inducing activation of V γ 9V δ 2 T cells or neither blocking activation nor inducing activation of V γ 9V δ 2 T cells. The immunoglobulins of the
10 invention are in particular for use in medical treatments and for use in assays involving V γ 9V δ 2 T cells.

Preferably, the immunoglobulin molecules according to the invention comprise a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 3, 6, 9, 11, 14, 17, 20, 22, 25, 27, 29,
15 30, 33, 35, 37, 40, 43, and 46. These CDR3 regions combined with the CDR1 and CDR2 sequences provided for binding and functions, as discussed in detail below.

In another aspect, there is provided a nucleotide sequence that encodes an immunoglobulin molecule as described herein.

In yet another aspect, there is provided a host cell comprising a nucleotide sequence
20 as described herein.

In yet another aspect, there is provided a method for preparing an immunoglobulin molecule as described herein, comprising:

- culturing a host cell as described herein;
- allowing the host cell to express the immunoglobulin;
- 25 - obtaining the immunoglobulin.

In yet another aspect, there is provided an immunoglobulin molecule as described herein for use in a medical treatment.

Also disclosed is a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that blocks activation of
30 human V γ 9V δ 2 T cells

Also disclosed is an immunoglobulin molecule as described herein for use in a medical treatment, wherein the medical treatment preferably comprises the use of inhibitors of the mevalonate pathway or wherein the medical treatment comprises the treatment of cancer or an infectious disease. Also disclosed is an immunoglobulin molecule as described
35 herein for blocking activation of human V γ 9V δ 2 T cells.

Also disclosed is immunoglobulin molecule as described herein, wherein the immunoglobulin molecule is an immunoglobulin molecule that activates human V γ 9V δ 2 T cells.

In yet another aspect, there is provided an immunoglobulin molecule as described herein
5 for use in a medical treatment, wherein preferably the medical treatment is a treatment of cancer.

In yet another aspect, there is provided non-therapeutic use of an immunoglobulin molecule as described herein for activating human V γ 9V δ 2 T cells.

In yet another aspect, there is provided an immunoglobulin molecule as described herein for activating human V γ 9V δ 2 T cells.

10 Also disclosed is an immunoglobulin molecule as described herein, wherein the immunoglobulin molecule is an immunoglobulin molecule that does not block activation of human V γ 9V δ 2 T cells; and does not activate human V γ 9V δ 2 T cells.

Also disclosed is the use of an immunoglobulin molecule as described herein for labelling
15 or for selecting human V γ 9V δ 2 T cells.

Figures:

Figure 1: Alignment of the VHH sequences wherein the framework regions (1, 2, 3 and 4) are indicated as well as CDR1, CDR2 and CDR3. The code for each of the VHHs is
20 indicated as well (i.e. 5C7 is the sequence of VHH 5C7).

Figure 2: VHH 5E7 and VHH 6F6 do not activate V γ 9V δ 2 T cells. Data indicate relative expression of the activation marker CD25, the pro-inflammatory cytokine IFN- γ , and the cytotoxic molecule granzyme B by healthy donor-derived V γ 9V δ 2 T cells in comparison with the positive control (phosphoantigen (pAg+) expressing HeLa cells)

25 Figure 3: VHH 5E7 neutralizes phosphoantigen induced activation of healthy donor-derived V γ 9V δ 2 T cells. A representative example demonstrates the dose dependent neutralization of phosphoAg-induced V γ 9V δ 2 T cell activation using VHH 5E7 while a non-specific VHH (negative control) cannot neutralize phosphoAg-induced V γ 9V δ 2 T cell activation. Vertical axis indicates activation of V γ 9V δ 2 T cells as assessed by CD25
30 expression, horizontal axis indicates different VHH concentrations. V γ 9V δ 2 T cell stimulations were performed using phosphoantigen expressing HeLa cells, generated by pretreating HeLa cells with increasing doses of the aminobisphosphonate pamidronate (which results in increasing levels of phosphoantigen expression by HeLa cells).

Figure 4: V γ 9V δ 2 TCR specific VHH are capable of inducing activation and cytokine
35 production in healthy donor-derived V γ 9V δ 2 T cells. Data indicate relative expression of the activation marker CD25 and the pro-inflammatory cytokine IFN- γ by healthy donor-derived V γ 9V δ 2 T cells in comparison with the positive control (phosphoantigen (pAg+) expressing

CLAIMS

1. A human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, comprising a CDR1 region, a CDR 2 region, and a CDR 3 region,
5 wherein the CDR1 region comprises an amino acid sequence with at least 40 % sequence identity with the amino acid sequence of SEQ ID NO. 31
GRTFSNYAMG, and
wherein the CDR2 region comprises an amino acid sequence with at least 60 % sequence identity with the amino acid sequence of SEQ ID NO. 32
10 AISWSGGSTYYADSVKG,
and
wherein the CDR3 region contributes to V γ 9V δ 2 T cell receptor binding; and
wherein the immunoglobulin molecule is an immunoglobulin molecule that
activates human V γ 9V δ 2 T cells.
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2. The immunoglobulin molecule according to claim 1, wherein the CDR2 region
comprises an amino acid sequence with at least 60 % sequence identity with SEQ
ID NO. 32 AISWSGGSTYYADSVKG, wherein the said amino acid sequence has a
T at position 9, an A at position 12, and a V at position 15.
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3. The immunoglobulin molecule according to claim 1 or claim 2, wherein the
immunoglobulin molecule is a single chain antibody, preferably wherein the single
chain antibody is a llama single chain antibody or a human single chain antibody.
- 25 4. The immunoglobulin molecule according to claim 1 or claim 2, wherein the
immunoglobulin molecule is a single domain antibody, preferably wherein the
single domain antibody is a llama single domain antibody or a human single
domain antibody.
- 30 5. The immunoglobulin molecule according to any one of claims 1-4, comprising one or
more of the framework regions selected from the group of amino acid sequences
of SEQ ID NO. 67-70.
6. A nucleotide sequence that encodes an immunoglobulin molecule according to any
35 one of claims 1-5.
7. A host cell comprising a nucleotide sequence according to claim 6.

8. Method for preparing an immunoglobulin molecule according to any one of claims 1-5, comprising:
- culturing a host cell according claim 7;
 - allowing the host cell to express the immunoglobulin;
 - obtaining the immunoglobulin.
9. An immunoglobulin molecule according to any one of claims 1-5 for use in a medical treatment.
10. The immunoglobulin molecule according to any one of claims 1-5, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 6, 9, 11, 14, 17, 20, 22, 25, 29, 33, 35, and 46.
11. The immunoglobulin molecule according to any one of claims 1-5 or claim 10, wherein the immunoglobulin molecule is linked to an agent.
12. An immunoglobulin molecule according to any one of claims 1-5, 10 or 11 for use in a medical treatment, wherein preferably the medical treatment is a treatment of cancer.
13. Non-therapeutic use of an immunoglobulin molecule according to any one of claims 1-5, 10 or 11 for activating human V γ 9V δ 2 T cells.
14. An immunoglobulin molecule according to any one of claims 1-5, 10 or 11 for activating human V γ 9V δ 2 T cells.