

The question, whether “fully human” mAbs such as derived from phage display libraries or transgenic mice are less immunogenic than humanized mAbs cannot be answered yet, because full immunogenicity data are available for just two human mAbs. An anti-tumor necrosis factor mAb, developed from phage-displayed human libraries induced antibody responses in 12% of patients - at the higher end of the incidence of anti-antibody responses of the humanized antibodies (Hwang, W. Y., et al. (2005). *Methods*, 36, 3-10).

Evaluation of the immunogenicity of the first registered human mAb generated by the transgenic approach demonstrated that mAb treatment resulted in the generation of antibodies in approximately 5.5% of treated cancer patients (Jakobovits, A., et al. (2007). *Nat. Biotechnol.* 25, 1134., Lofgren, J. A., et al. (2007). *J. Immunol.* 178, 7467).

A need therefore remains for a method and means for producing antibodies that are specific for their targets, but are less immunogenic. According to the invention the reduction of immunogenicity is at least partially achieved by providing a transgenic non-human mammal comprising, at least in its B cell lineage, a nucleic acid encoding at least an immunoglobulin light chain or heavy chain, wherein the heavy- or light chain encoding sequence is provided with a means that renders it resistant to DNA rearrangements and/or somatic hypermutations, preferably such a non-human animal is a rodent, more specifically a mouse. The nucleic acid preferably encodes a human, human-like or humanized immunoglobulin chain.

Summary

In one aspect, the present disclosure provides a transgenic murine mammal comprising in its genome a rearranged nucleic acid encoding a rearranged human immunoglobulin light chain variable region, wherein the rearranged human immunoglobulin light chain variable region is capable of pairing with at least two different heavy chains encoded by the murine mammal, such that variety in specificity of antibodies is retained through rearrangements and hypermutations in the heavy chains, for the generation of high affinity antibodies comprising said rearranged human immunoglobulin light chain variable region, wherein the

antibodies are encoded by VH genes that have undergone somatic hypermutation upon immunization and the non-mutated rearranged human VL gene integrated in the genome.

5 In another aspect, the present disclosure provides a method for the production of a transgenic murine mammal, comprising the step of introducing a rearranged nucleic acid encoding a rearranged human immunoglobulin light chain variable region into the murine mammal germline, wherein the rearranged human immunoglobulin light chain variable region is capable of pairing with at least two different heavy chain variable regions encoded by the murine mammal, such that variety in specificity of antibodies is retained through rearrangements and hypermutations in the heavy chains, for the generation of high affinity antibodies comprising said rearranged human immunoglobulin light chain variable region, wherein the antibodies are encoded by VH genes that have undergone somatic hypermutation upon immunization and the non-mutated rearranged human VL gene integrated in the genome.

15 In yet another aspect, the present disclosure provides a method for producing a desired antibody comprising exposing a transgenic murine mammal as described herein to an antigen such that an antibody response is induced and isolating antibodies specific for the antigen.

20 In yet another aspect, the present disclosure provides a method for producing a desired antibody comprising exposing a transgenic murine mammal as described herein to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and harvesting said antibodies.

25 In yet another aspect, the present disclosure provides a method for producing a desired antibody comprising exposing a transgenic murine mammal as described herein to an antigen such that an antibody response is induced and isolating a VH gene which has undergone somatic hypermutation and which encodes the VH of a high affinity antibody generated, which comprises the rearranged human immunoglobulin light chain variable region, inserting said isolated VH gene or a copy thereof in an expression cassette and expressing said high affinity antibody in a host cell.

In the remainder of the specification mice are typically used as examples of the non-human mammals. The transgenic non-human mammalian hosts are capable of mounting an immune response to an antigen, where the response produces antibodies having primate, particularly human, variable regions. Various transgenic hosts may be

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Claims

1. A transgenic murine mammal comprising in its genome a rearranged nucleic acid encoding a rearranged human immunoglobulin light chain variable region,
 5 wherein the rearranged human immunoglobulin light chain variable region is capable of pairing with at least two different heavy chains encoded by the murine mammal, such that variety in specificity of antibodies is retained through rearrangements and hypermutations in the heavy chains, for the generation of high affinity antibodies comprising said rearranged human immunoglobulin light chain variable region,
 10 wherein the antibodies are encoded by VH genes that have undergone somatic hypermutation upon immunization and the non-mutated rearranged human VL gene integrated in the genome.
2. The transgenic murine mammal according to claim 1, wherein the rearranged
 15 nucleic acid encoding a rearranged human immunoglobulin light chain variable region further comprises an immunoglobulin constant light chain region.
3. The transgenic murine mammal according to claim 1, wherein the rearranged
 20 nucleic acid encoding a rearranged human immunoglobulin light chain variable region is operatively linked to an endogenous immunoglobulin constant light chain region.
4. The transgenic murine mammal according to any one of claims 1-3, which is a mouse.
- 25 5. A method for the production of a transgenic murine mammal, comprising the step of introducing a rearranged nucleic acid encoding a rearranged human immunoglobulin light chain variable region into the murine mammal germline,
 wherein the rearranged human immunoglobulin light chain variable region is capable of pairing with at least two different heavy chain variable regions encoded by the murine
 30 mammal, such that variety in specificity of antibodies is retained through rearrangements and hypermutations in the heavy chains, for the generation of high affinity antibodies comprising said rearranged human immunoglobulin light chain variable region,

wherein the antibodies are encoded by VH genes that have undergone somatic hypermutation upon immunization and the non-mutated rearranged human VL gene integrated in the genome.

5 6. A method for producing a desired antibody comprising exposing a transgenic murine mammal according to any one of claims 1-4 to an antigen such that an antibody response is induced and isolating antibodies specific for the antigen.

10 7. A method for producing a desired antibody comprising exposing a transgenic murine mammal according to any one of claims 1-4 to an antigen such that an antibody response is induced and isolating cells producing such antibodies, culturing and harvesting said antibodies.

15 8. The method according to claim 7, further comprising immortalizing said cells after the culturing step and prior to harvesting said antibodies.

20 9. A method for producing a desired antibody comprising exposing a transgenic murine mammal according to any one of claims 1-4 to an antigen such that an antibody response is induced and isolating a VH gene which has undergone somatic hypermutation and which encodes the VH of a high affinity antibody generated, which comprises the rearranged human immunoglobulin light chain variable region, inserting said isolated VH gene or a copy thereof in an expression cassette and expressing said high affinity antibody in a host cell.

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