A novel approach for generation of fully human monoclonal antibodies in humanized RAG-hu mice

Abstract # 4090

A. Lifke, V. Lifke, B. Bossenmaier, H.W. Krell, T. Schnitzer , B. Mueller-Beckmann
Roche Diagnostics GmbH, Pharma Research, Penzberg, Germany

Abstract: **The RAG-hu mouse model has proven to be suitable for generation and future immortalization of human B cells specific for disease-associated human antigens.**

Introduction

The clinical use of murine monoclonal antibodies is limited by the human anti-murine antibody responses induced in patients. The currently well-established methods for developing fully human mAbs are human hybridoma technology, transgenic mice and phage display [1]. Common limitations of these approaches are low affinity, restricted Ab diversity or cross-reactivity.

A Human adaptive immune system in a cord blood cell–transplanted humanized Rag2-/-gamma c-/- mouse model (RAG-hu) sustains multi-lineage human hematopoiesis and is capable of mounting immune responses [2].

In the present study we demonstrate that an immundeficient non-human animal, reconstituted with human fetal liver stem cells is suitable for developing of human therapeutic mAbs against e.g. cancer, autoimmune or infectious diseases.

1. Reconstitution	2. Immunization	3. Fusion

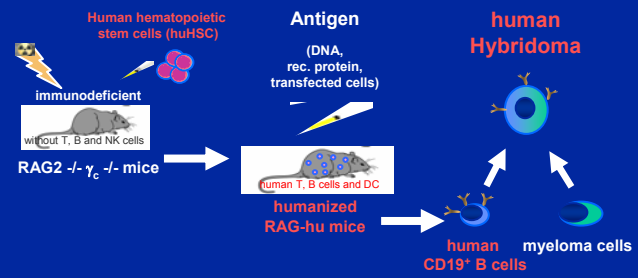


Figure 1. The process of generation of human mAb´s in RAG-hu mouse model consists of three main steps. 1. Establishment of human adaptive immune system in immunodeficient mice via reconstitution of RAG2, common gamma chain double knock out mice with human hematopoietic stem cells. 2. Challenge of the established human´s adaptive immune system with antigen. 3. Fusion of extracted human B cells with myeloma cells.

Materials and Methods

RAG2-/- γ_c-/- mice were originally kindly provided by RCMG, Basel, Switzerland and thereafter were breed in Roche´s animal facility.
Human hematopoietic stem cells (huHSC) were obtained from Cambrex Corporation, New Jersey, USA. Reconstitution of immunodeficient animals with huHSC was done according the protocol described in [2].
The reconstitution status of transplanted animals were analyzed by FACS. Peripheral blood was collected and stained with FITC labeled hu-CD45-specific or control mAb´s (Pharmingen) according to the manufacture's protocol.
Serum was collected by retro-orbital bleeding and the level of total or antigen-specific human IgG was assessed by sandwich ELISA. The same test was applied for the detection of antigen-specific human mAb´s in supernatant from primary human hybridomas.
Human B cells were isolated from the spleen of immunized RAG-hu animals via EasySep® Human B Cell Enrichment Kit (StemCell Technologies Inc) and electrofused using the Eppendorf Multiparator according to the manufacture's protocols.

Results

To asses the reconstitution level of huHSC transplanted RAG-hu mice flow cytometric analysis for human leucocytes in peripheral blood of these animals was performed.

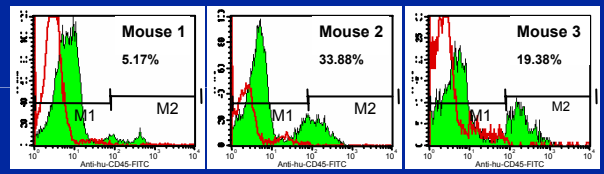


Figure 2. Flow cytometric detection of human CD45+ leucocytes in the peripheral blood of reconstituted RAG-hu mice 15 weeks after engraftment. Background staining with isotyp matched control mAb is shown in red.

To evaluate the capacity of RAG-hu mice to generate antigen-specific human B cells, they were challenged with either a mixture of antigenic proteins plus adjuvant or with plasmid DNA encoding the target antigen. The specific humoral response was assessed by ELISA and FACS. Our results show that both protein and genetic immunizations are capable of generating antigen-specific human B cells in RAG-hu mice. The specific titer rises depending on boost injection and lasts for at least 20 weeks.

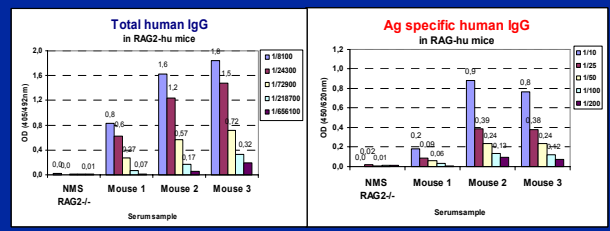
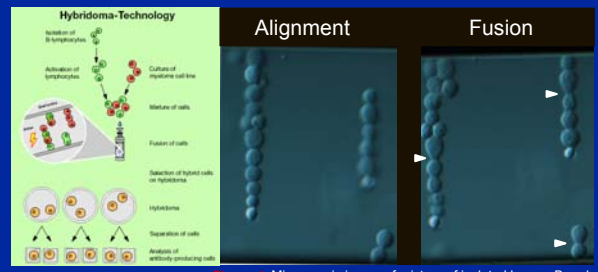


Figure 3. Biochemical detection of total and antigen-specific human IgG in serum of RAG-hu mice at d100 after immunization. Background signal was evaluated with serum from non-reconstituted RAG2-/- γ_c-/- animals.

Human B cells were isolated from spleen of immunized RAG-hu mice and used for hybridoma generation via electrofusion.



Source: http://www.eppendorf.de/de/index.php

Figure 4. Microscopic image of mixture of isolated human B and myeloma cells in the micro fusion chamber.

Rescreening of primary human Hybridomas

Object Id	Ag ELISA C (µg/ml)	irrelevant Ag ELISA c(µg/ml)
huXXX_M03.03B3:1	0,29	<0
huXXX_M03.02B5:1	0,24	<0
huXXX_M03.01E2:1	0,20	<0
huXXX_M03.01E7:1	0,13	<0
huXXX_M03.01G4:1	0,09	<0
huXXX_M02.01G2:1	0,02	<0
huXXX_M02.01G3:1	0,17	<0
huXXX_M02.02C10:1	0,55	<0

Table 1. Summary of rescreening of primary human hybridomas generated from human B cells isolated from two immunized RAG-hu animals. Biochemical detection of antigen-specific mAb´s in the supernatant of human hybridomas (in red). Binding specificity of generated human mAb´s was assessed by ELISA with irrelevant control protein as antigen (in green).

Conclusions

The successful generation of hybridomas producing fully human antigen-specific monoclonal antibodies from isolated human B cells derived from immunized mice transplanted with human hematopoietic stem cells indicates that the humanized RAG-hu mouse model could provide a novel tool for developing completely non imunogenic mAbs for therapeutic or diagnostic use.

The considerable advantage of this approach is the possibility to generate a more diverse panel of mAbs with a broad range of characteristics, because the use of entire human B cells repertoire as well as other cellular components of the human immune system and other biomolecules required for antibody development.

Therefore, the humanized RAG-hu mouse model is a promising approach for future immunotherapy development.

[1] Brekke OH, Sandlie I. "Therapeutic antibodies for human diseases at the dawn of the twenty-first century." ***Nat Rev Drug Discov. 2003 Jan;2(1):52-62.***

[2] Traggiai E. et. al, "Development of a human adaptive immune system in cord blood cell-transplanted mice." ***Science. 2004 Apr 2;304(5667):104-7.***

For more information contact alexander.lifke@roche.com

Acknowledgment

The authors thank to M. Kopp, I. Wuensche, A. Lutz, U. Kolb, M. Friedrich and S. Kettenbach for excellent technical support.

AACR, April 2007 Los Angeles, CA