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Review

# Critical assessment of human antibody generation in humanized mouse models

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Immunodeficient mice reconstituted with human hematopoietic stem cells provide a small-animal model for the study of development and function of human hematopoietic cells in vivo. However, in the current models, the immune response, and especially the humoral response by the human immune cells is far from optimal. The B cells found in these mice exhibit an immature and abnormal phenotype correlating with a reduced capacity to produce antigen-specific affinity matured antibodies upon infection or immunization. Herein, we review the current state of knowledge of development, function and antibody production of human B cells and discuss the obstacles for the improvement of these models.

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## 1. Introduction

Over the years monoclonal antibodies (mAbs) have repeatedly proven their efficacy for clinical use in a wide range of conditions including viral and bacterial infections (Ter Meulen, 2011), auto-immunity and inflammation (Chan and Carter, 2010), as well as the induction of anti-tumor responses (Sliwkowski and Mellman, 2013). Due to their high selectivity mAbs represent interesting options to target the treatment to cells of interest with low off-target toxicity. Today several mAbs are available for clinical use and many more are under development.

It has become clear that due to immunogenicity of xenogeneic proteins, the first generation of mouse mAbs was not very effective in humans (Sgro, 1995). Humanization of these antibodies – by replacing immunogenic domains in the murine backbone with their human counterparts – greatly diminished the immune reaction against the antibody, thereby significantly enhancing their clinical effectiveness. However the generation of fully human antibodies would alleviate the need for the complex process of humanizing murine antibodies.

To address this problem several generations of transgenic mice bearing the human immunoglobulin-encoding gene segments were developed to obtain fully human antibodies upon immunization (Lee and Owen, 2012). Although this technique has successfully produced clinical antibodies, the generation of such mouse strains remains challenging, as illustrated by abnormalities in the B cells development and antibodies production observed in the initial models. A new generation of transgenic mice bearing human immunoglobulin seems to hold more promises but only limited data detailing these models are currently available (Lee and Owen, 2012) and their use is limited by several patents.

B cells collected from humans represent an optimal source to obtain relevant fully human mAbs. It is now possible to efficiently immortalize antibody-producing B cells from human blood (Traggiai et al., 2004a; Kwakkenbos et al., 2013). Immortalization of human B cells proved to be very efficient in generating fully human antibodies against viruses and bacteria. However, it is much more difficult to get antibodies against human proteins because the B cell system is tolerant for human proteins that have been encountered during development of these cells.

Over the past decades, a small-animal model to study development and function of human hematopoietic cells has been developed (Shultz et al., 2012; Rongvaux et al., 2013). Injection of human hematopoietic stem/progenitor cells (HSPCs) in immunocompromised mice results in the de novo development of innate and adaptive immune cells. Several strains of immunocompromised mice efficiently supporting the engraftment of human cells and tissues from different origins (fetal tissues, umbilical cord blood, mobilized blood, bone marrow) have been developed over the years. Mutations in or deletion of the interleukin-2 receptor common gamma chain (IL-2Rγ) combined either with Rag1 or Rag2 gene ablation (BALB/c $Rag2^{-/-}$IL-2$R\gamma^{-/-}$, called BRG; NOD $Rag2^{-/-}$IL-2$R\gamma^{-/-}$, called NRG) or with a SCID mutation (NOD-SCID IL-2$R\gamma^{-/-}$, NSG or NOG) provides highly immunocompromised hosts suited for the engraftment of human cells both in newborns and in adult mice. Two types of humanized mice are widely used today. Human Immune System (HIS) mice are generated by the injection of human HSPCs in BRG (Gimeno et al., 2004; Traggiai et al., 2004b; Legrand et al., 2011) or NSG/NOG mice (Ito et al., 2002; Ishikawa et al., 2005; Shultz et al., 2005). Another model comprises mice engrafted with human fetal liver and thymus under the kidney capsule, followed by injection of HSPCs isolated from the liver of the same donor. These mice are called BLT mice (Lan et al., 2006; Melkus et al., 2006). In both the HIS and BLT mouse systems, cells from the different human hematopoietic lineages, including B and T cells, are present in primary and secondary lymphoid organs, as well as in peripheral blood of the mice. The specific advantages of each model have been recently reviewed elsewhere (Drake et al., 2012; Ito et al., 2012; Shultz et al., 2012; Rongvaux et al., 2013). However, despite several reports demonstrating that human IgG can be produced in humanized mice, the scarcity of class-switched matured antibodies limits studying the physiological humoral response to infections and vaccines. In this review, we focus on the development of human B-cell antibody production and affinity maturation in HIS and BLT mice as compared to the physiological human situation.

## 2. B-cell development is a strictly controlled multistep process

### 2.1. Development of naïve human B cell in the human bone marrow

In the bone marrow, hematopoietic stem cells differentiate through a series of sequential stages to mature B cells. During the early stages of B-cell differentiation diversity of the B-cell receptor (BCR) is acquired through recombination of multiple segments of the Ig locus. RAG-1 and RAG-2 are the two enzymes that catalyze the recombination of the variable, diversity, and junction (V, D, J) segments by non-homologous end joining. Additional diversity is gained by random insertion of nucleotides at the joining exons, a process mediated by the enzyme terminal ribonucleotidyl transferase (TdT). By these recombination and insertion events every B cell produces a unique BCR on its cell surface capable of recognizing antigen (Fig. 1).

During early B-cell development different sequential developmental stages can be recognized. The current consensus is that human B lineage-restricted cells pass through the following stages; early B, pro-B, pre-BI, large pre-BII and, small pre-BII cells (Blom and Spits, 2006). These differentiation steps are linked to the expression of specific cell surface markers and levels of the components to the BCR recombination machinery (Ghia et al., 1996). During the early B cell stages D–J rearrangement of the Ig heavy chain is initiated (Bertrand et al., 1997). Subsequently pro-B cells proceed to rearrange the V–$DJ_H$ loci (Bertrand et al., 1997; Davi et al., 1997). Once V–$DJ_H$ rearrangement is complete and resulted in the production of an in-frame IgH protein, the Igμ is expressed on the cell surface in complex with the invariant surrogate light chain proteins VpreB and λ5. VpreB and λ5 substitute for the Ig light (IgL) chain whose loci have not yet been rearranged (Löffert et al., 1996). Surface expression of the pre-BCR marks the large pre-BII cellular stage and is a key checkpoint in B cell development to test for functionality of the produced heavy chain. Positive selection through the pre-BCR confirms that the

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