Development of immunogenicity models in mice for improved risk assessment of biopharmaceuticals

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av

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This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Granath, B. Holgersson, J. Brenden, N. Refined analysis of antigen-specific antibody responses- A new one-step tool in immunogenicity studies. European Journal of Pharmaceutical Sciences 44 (2011) 187-193.

II. Brenden, N. Madeyski-Bengtson, K. Martinsson, K. Svärd, R. Albery-Larsdotter, S. Granath, B. Lundgren, H. Lövgren, A. A Triple Transgenic Immunotolerant Mouse Model. Journal of Pharmaceutical Sciences, Vol. 102, No. 3, March 2013.

III. Granath, B. Holgersson, J. Cederbrant, K. Brenden, N. A Comparison of the humoral immune response induced by a recombinant human protein in wild type mice and in transgenic mice expressing the protein. (Manuscript).

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Abstract

The development of anti-drug-antibodies (ADA) to biopharmaceuticals, *e.g.* recombinant proteins including monoclonal antibodies (mAb) can lead to adverse events and clinical complications. These include reduced effect of the drug and autoimmune conditions if the administered drug is analogous to endogenous proteins. Immunogenicity assessment is critical during biopharmaceutical development and evidence for possible immunogenicity is required before drug approval.

In this thesis, we have focused on improving the assessment of immunogenicity to human recombinant protein drugs using *in vivo* mouse models in order to I) develop an *ex vivo* screening-method that can detect, characterize and semi-quantify specific ADA in one single plasma sample; II) characterize the differences in immune responses to a human recombinant protein drug-candidate with respect to ADA class- and subclass profiles, between wild type (Wt) and transgenic (Tg) mice expressing the human protein; III) investigate subclass profiling of ADA responses in Wt- and Tg-mice and to correlate the drug-specific subclasses with mechanisms for ADA production; IV) generate an immune-tolerant mouse model expressing human coagulation factors II, VII and X to be used for process optimization of biopharmaceuticals; V) correlate the ADA response in mouse models with the quality of batch formulations, *e.g.* presence of degradation products, drug fragments, endotoxins and host-cell-proteins, known as potential contributors to increased immunogenicity; VI) work towards the principles of "3R".

The results indicate that the developed *ex vivo* immunogenicity assay can detect immunoglobulin (Ig) subclasses of ADA with high specificity and sensitivity (125 ng/ml) in one single sample. Further, we saw a connection between low batch purity and high ADA levels. The least pure batch induced a significant increase in ADA of subclass IgG1 in both Wt- and Tg-mice. Since the Tg-mice were supposed to be tolerant to immunization with the human protein itself, the impurities (fragments, degradation products, endotoxins and more), included in the formulation, likely caused broken tolerance and subsequent ADA-formation in these animals. Wt-mice also showed IgG2b responses in a majority of the animals compared to none of the Tg-mice. It is suggested that the IgG2b response in Wt-mice is an expression of a xeno-response to the human protein. The combination of IgG1 and IgG2b in Wt-mice was reflected by a Th2-related cytokine repertoire in plasma. Finally, the developed triple transgenic mouse model expressing human coagulation factors II, VII and X, showed only low titers of ADA after immunization with pure drug formulation. Therefore, this model will be valuable during process optimization in order to monitor a potential ADA response.

By developing an assay for detection of subclasses of ADA, we have enabled the monitoring of immunogenicity in pre-clinical studies in a new way. By implementing the use of immune tolerant mouse models, commonly used for product quality assessment, we have contributed to reduce the use of animals and at the same time added tools for better risk assessment of immunogenicity.

Keywords: anti-drug antibodies, immunogenicity, biopharmaceuticals, immunoglobulin, 3R

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