INTRODUCTION

Immunogenicity of biopharmaceutical products is a major concern in both pre-clinical and clinical studies. Reliable validated assays for detection and characterization of anti-drug antibodies (ADA) are required. Beside screening and confirmatory assays, ADA-isotyping provides additional information that is important for the understanding of a particular immune response. In order to characterize immunogenicity of a drug (a serum protein, MW ca 50 KDa) in human, specific assays for both IgG and IgM ADAs were developed in our laboratory. For comparison of relative IgG and IgM responses, ADA-positive human plasma samples were subjected to both assays.

ANALYTICAL METHODS

The immunogenicity-assessment strategy adopted in our laboratory included a screening assay, followed by specificity confirmation in a confirmatory assay. Positive samples were subjected to a titration assay for relative quantification. Further characterization included IgG, and IgM isotype detection and titration (Fig 1). IgE ADA detection and ADA relative affinity determination were also performed but are not presented here.

DEVELOPMENT OF ISOTYPING ASSAYS

The critical steps of assay development included:

1. Determination of best conditions for reducing unspecific binding
2. Determination of the minimum required dilution (MRD)
3. Adaptation of test concentrations of detection antibodies
4. Detection of IgM ADA in presence of high titers of IgG
5. Strategy for cut point calculation adapted to variation between individuals

High background was a challenge successfully addressed by choosing suitable blocking conditions. Best results were obtained with 2% Casein blocking buffer. Best sensitivity and reduction of matrix effects were achieved with an MRD of 1:50 for both assays. Dilutions of detection antibodies were optimized for each test (1:1000 and 1:100 000 for IgM and IgG, respectively).

The ability to detect specific IgM ADA in presence of high titers of IgG was investigated in 2 assay formats. Clinical samples were included in method development and tested with either drug or anti-human IgM capturing format. Samples taken at three time points (0, 30 and 90 days post treatment) from patients subjected to a single dosage were used (Fig 7). A clear IgG interference was not detected. However, the format with drug as capture reagent showed better sensitivity and was chosen (Fig 7A and 7B). Therefore, this assay format was used for validation and sample analysis.

DEVELOPMENT OF ISOTYPING ASSAYS

Sample specific cut points were calculated to take into consideration the observed variations between individuals. The adequacy of this approach was confirmed during validation.

VALIDATION OF ISOTYPING ASSAYS

IgG and IgM assays were successfully validated based on current ADA validation standards. Fifty individual samples from healthy subjects were repeatedly tested to determine validation cut point and correction factor (Fig 8).

DISCUSSION AND CONCLUSION

The design of surrogate positive control fusions permitted the development and validation of ADA-isotyping assays to be used for analysis of human samples.

The isotyping assays presented here were shown to be sensitive, precise, specific and robust. It was demonstrated that low levels of IgM ADA could be detected even in the presence of high titers of IgG ADA.

REFERENCES


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