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Isolation of high-affinity, neutralizing anti-idiotype antibodies by phage and ribosome display for application in immunogenicity and pharmacokinetic analyses

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ABSTRACT

Anti-idiotype antibodies against a therapeutic antibody are key reagents for the development of immunogenicity and pharmacokinetic (PK) assays during pre-clinical and clinical development. Here we have used a combination of phage and ribosome display to isolate a panel of monoclonal anti-idiotype antibodies with sub-nanomolar affinity and high specificity to a human anti-IgE monoclonal antibody. Anti-idiotype antibodies were enriched from scFv libraries using phage display, and a biochemical epitope competition assay was used to identify anti-idiotypes which neutralized IgE binding, which was essential for the intended use of the anti-idiotypes as positive controls in neutralizing anti-drug antibody (Nab) assays. The phage display-derived anti-idiotype antibodies were rapidly affinity-matured using a random point mutagenesis approach in ribosome display. Ten anti-idiotype antibodies with improved neutralizing activity relative to the parent antibodies displayed sub-nanomolar affinity for the anti-IgE antibody, representing up to 20-fold improvements in affinity from just two rounds of affinity-based selection. The optimized anti-idiotype antibodies retained the specificity of the parent antibodies, and importantly, were fit for purpose for use in PK and anti-drug antibody (ADA) assays. The approach we describe here for generation of anti-idiotype antibodies to an anti-IgE antibody is generically applicable for the rapid isolation and affinity maturation of anti-idiotype antibodies to any antibody-based drug candidate.

1. Introduction

Monoclonal antibody (mAb) therapeutics are an important and rapidly growing sector of the pharmaceutical industry, with 28 marketed products in the US and Europe (Reichert, 2012), and hundreds more in pre-clinical or clinical development (Nelson et al., 2010). Two critical elements in the clinical development of monoclonal antibodies are the detection of anti-drug immune responses and the measurement of pharmacokinetic (PK) profiles in the patient population. Assays for both types of measurements require reagents, which possess high affinity and specificity for the therapeutic mAb, such as anti-idiotype antibodies.

The development of immunogenicity assays to measure anti-drug antibodies (ADA) is a regulatory requirement (EMEA guidance, 2007; FDA draft guidance, 2009) as an anti-drug immune response may affect drug safety and efficacy. Typically, immunogenicity screening assays are performed in a bridging format, with the therapeutic antibody used for capture and detection of ADA in patient serum (Mire-Sluis et al., 2004; Shankar et al., 2008). Anti-idiotype antibodies, which bind to the variable region of the therapeutic antibody, are used as positive controls, serving as the standards by which ADA assay

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performance is judged. Neutralizing anti-idiotype antibodies, which antagonize antigen binding by the therapeutic antibody, are often required for use as positive controls in neutralization assays to measure any neutralizing anti-drug antibodies (Nabs) in patients exhibiting an ADA response (Gupta et al., 2007; Koren et al., 2008).

Pharmacokinetic assays are used to quantify therapeutic antibody levels in serum samples during pre-clinical and clinical trials, providing drug exposure information and ultimately, guidance on dosage and dosing frequency (Mahmood and Green, 2005). PK assays require specific and high-affinity reagents for capture of the therapeutic mAb from patient serum. When a PK assay is intended to measure free or partially target-bound drug, both antigen and neutralizing anti-idiotype antibodies are generally suitable as capture reagents. However, anti-idiotype antibodies may offer certain advantages over antigen, including superior stability, lower cost and greater ease and reliability of production (Roskos et al., 2011). Thus, the use of a monoclonal anti-idiotype antibody rather than antigen for PK assays ensures that a quality reagent is available for the lifetime of the project. Furthermore, non-neutralizing anti-idiotype antibodies, but not antigen, can be used as capture reagents when a PK assay is intended to measure total drug.

Anti-idiotype antibodies must fulfill a number of requirements for use in PK and ADA assays (Staack et al., 2011). Assay sensitivity, or limit of detection (LOD), is a particularly crucial consideration. To ensure detection of all clinically relevant anti-drug antibodies, the FDA requires an assay sensitivity of 250–500 ng/ml for ADA assays (Mire-Sluis et al., 2004; FDA draft guidance, 2009). For PK assays (Desilva et al., 2003), the lower limit of quantification (LLOQ) required varies based on the projected drug concentration profile in a particular study. LLOQ of a PK assay for a mAb is generally preferred to be less than 100 ng/ml, although in preclinical animal studies where animals are typically dosed at high levels, an assay with a higher LLOQ may be fit for purpose. In order to achieve the required levels of sensitivity in PK and ADA assays, anti-idiotype antibodies generally must possess high (often sub-nanomolar) affinity for the therapeutic antibody (Liang et al., 2007). Anti-idiotype antibodies must also possess exquisite specificity for the therapeutic antibody, as patient serum samples will contain large populations of irrelevant antibodies.

Although polyclonal anti-idiotype antibodies are widely used for ADA assays, monoclonal anti-idiotype antibodies offer a number of advantages for this purpose. Monoclonal anti-idiotypes will have greater and more clearly defined specificity for the therapeutic mAb than polyclonal anti-idiotypes. A panel of monoclonal anti-idiotypes with a range of affinities for the therapeutic mAb can be used to verify that an ADA assay detects anti-drug antibodies with both high and low affinity for the therapeutic antibody (Liang et al., 2007), which is not possible with polyclonal anti-idiotype antisera. A neutralizing monoclonal anti-idiotype antibody will also be a more reliable positive control in neutralization assays, as neutralizing polyclonal antisera may also contain antibodies with alternative epitope specificities. Furthermore, monoclonal anti-idiotype antibodies have a clear advantage over polyclonal antibodies in terms of superior reliability and reproducibility of manufacture, which is critical given long clinical development timelines.

However, the identification of high-affinity, specific monoclonal anti-idiotype antibodies suitable for ADA and PK assays is not trivial. Typically, anti-idiotype antibodies are derived from traditional hybridoma methods (Kohler and Milstein, 1975). However, phage display has recently emerged as a viable alternative for the generation of anti-idiotype antibodies (Goletz et al., 2002; Tornetta et al., 2007; Ylera et al., 2013). Phage display is a robust method, which enables identification of human anti-idiotype antibodies from naïve scFv or Fab libraries in timeframes compatible with preclinical ADA and PK assay development. Selection conditions and/or screening assays can be designed to facilitate identification of anti-idiotype antibodies with desired properties, such as a particular epitope specificity (e.g., neutralizing or non-neutralizing). However, because selections are generally performed with naïve antibody libraries, the anti-idiotype antibodies generated from phage display selections may not have sufficiently high affinity to deliver the sensitivity required for ADA and PK assays (Tornetta et al., 2007). In these cases, affinity maturation of lead anti-idiotype antibodies is required.

Ribosome display is a cell-free platform where genotype (mRNA) and phenotype (antigen binding) are coupled through formation of stable ternary mRNA–ribosome–protein complexes (Plückthun, 2012). Iterative cycles of affinity-based selection and PCR amplification are used to enrich proteins with improved affinity from libraries of protein variants. Ribosome display is an ideal method for affinity maturation of anti-idiotype antibodies due to the ease with which large ($>10^{12}$) libraries of point mutants can be constructed and improved variants rapidly selected (Groves and Nickson, 2012; Lewis and Lloyd, 2012).

The aim of this study was to identify high-affinity, neutralizing anti-idiotype antibodies against a human anti-IgE therapeutic antibody (MEDI4212), which would be suitable for use in ADA and PK assays during pre-clinical and clinical development. Phage display was used to isolate anti-idiotype antibodies against MEDI4212 from human scFv libraries, and the lead anti-idiotypes were then rapidly affinity matured using affinity-based selections in ribosome display to enrich for improved variants from libraries of random point mutants. This approach, which is generically applicable to anti-idiotype antibody discovery for any antibody, delivered an anti-idiotype antibody against MEDI4212, which is fit for purpose for use in both ADA and PK assays.

2. Materials and methods

2.1. Antibodies and reagents

MEDI4212 is an anti-IgE human IgG1 antibody in pre-clinical development for asthma. GLM1 is a human IgG1 antibody against an irrelevant antigen, which is germline-matched to MEDI4212 in both the heavy (IMGT: IGHV1-f*01) and light (IMGT: IGLV1-40*01) chains. MEDI4212 and GLM1 were produced in HEK-EBNA or GS-CHO cells and purified by affinity chromatography on protein A or protein G (for selections). Anti-idiotype IgG4 antibodies were produced in HEK-EBNA cells and purified by affinity chromatography on protein A. All scFv antibodies were expressed in TG1 *Escherichia coli* and purified via the C-terminal His tag by immobilized nickel chelate chromatography (Bannister et al., 2006). IgG and scFv antibodies were biotinylated by standard amine coupling chemistry. Free biotin was removed by size exclusion chromatography, and biotin incorporation confirmed by MALDI-TOF.