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Generation and Detailed Characterization of an Affinity Purified Polyclonal Chicken IgY to Human IgG with Negligible Cross-Reactivity to Monkey IgG

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PURPOSE

Well characterized, high-quality critical reagents are increasingly important for ligand binding assays (LBA) supporting biotherapeutic development. Preclinical pharmacokinetic (PK) and toxicokinetic (TK) studies are often performed in non-human primates. Here we present a strategy for producing a highly purified chicken IgY antibody against human IgG (the basis of therapeutic mAbs) in matrices containing monkey serum.

METHOD(S)

Antibody Purification and Conjugation.

Chickens were immunized with highly purified monomeric human total IgG from myeloma plasma. Following ELISA confirmation of immune response, eggs were collected and refrigerated. Total IgY was precipitated from homogenized yolks using increasing concentrations of polyethylene glycol (PEG), up to 15% w/v. Pelleted IgY was resuspended in phosphate buffered saline (PBS), acidified, diluted 5-fold, and refrigerated overnight without stirring. Demulsified lipids were skimmed off before filtration and concentration by tangential flow filtration (TFF). Concentrated IgY was precipitated using ammonium sulfate, up to 41% saturation (1.6 M), on ice with gentle stirring. Pelleted IgY was re-dissolved in PBS. Cross-reactivity was depleted using clarified serum from rat, rabbit, cynomolgus monkey, and African Green monkey, with 4% w/v PEG to accelerate complexed antibody precipitation. This preparation was purified by immunoaffinity chromatography using immobilized human IgG in elution mode, then monkey IgG in flow-through mode. Biotinylated (Bt-IgY) and ruthenylated (Ru-IgY) were prepared using commercial NHS ester derivatives, moderate protein:tag ratios (1:10 to 1:15), and alkaline buffer (pH 7.5-8.5). Conjugates were desalted to remove free tag and characterized using SDS-PAGE, ECLIA, and BLI.

ECLIA (MSD) Method.

Immunoglobulin (Ig) classes, IgG subclasses, and Ig light chains were serial diluted (1:2) in PBS. MSD plates were passively coated at 4 °C overnight (> 16 hours, 50 µL sample/well). Plates were washed with TBST. Ru-IgY was diluted to 1 µg/mL in Assay Buffer, 50 µL was applied to wells. Plates were incubated at RT for 30-60 minutes with shaking. After the final wash, 150 µL of MSD Gold Read Buffer A was applied to each well. Plates were read using an MSD SQ120 MM plate reader within 10 min. SigmaPlot was used for 5-PL regression and EC_{50} calculation.

BLI (Octet) Method.

Bt-IgY(5 µg/mL) and human antibody standards were diluted in Sartorius Assay Buffer. 180 µL/well (96-well plate) or 100 µL/well (384-well plate) were added to F-bottom plates. Streptavidin biosensors were hydrated (Assay Buffer > 10 min., 30 °C). Assays were run at 30 °C and 1,000 RPM. The instrument sequentially moved streptavidin biosensors through the following phases: Baseline (Assay Buffer, 60 seconds), Loading (Bt-IgY for 120 seconds), Baseline (Assay Buffer, 120 seconds), Association (IgG solution, 300 seconds), Dissociation (Assay Buffer, 300-600 seconds). Assay blanks were subtracted from raw signal for each sample, and baselines were aligned from the last 5 seconds of Baseline 2. Curve fitting used appropriate binding models, based on X^2 and R^2, to quantitate binding strength.

Figure 1. Confirmation of depleted cross-reactivity by ELISA method. At 1 : 10,000 dilution factor, the chicken IgY shows negligible signal against wells coated with rabbit, rat, or monkey IgG but robust signal (> 3 AU) against human IgG.
Note: * = maximal signal response.

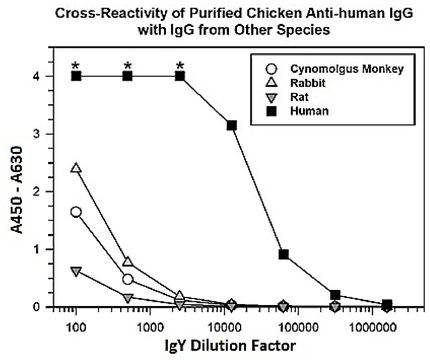


Figure 2. (A) SDS-PAGE of biotinylated and ruthenylated chicken IgY under non-reducing and reducing conditions. M = molecular weight ladder; i = IgY standard; ii = Unreduced Bt-IgY; iii = Unreduced Ru-IgY; iv = Reduced Bt-IgY; v = Reduced Ru-IgY; vi = Reduced IgY Standard. (B) MSD assay of ruthenylated anti-human antibodies in presence of 1% monkey sera, (C) MSD assay of biotinylated anti-human antibodies in presence of 1% monkey sera using Ru-conjugated streptavidin as detection reagent.

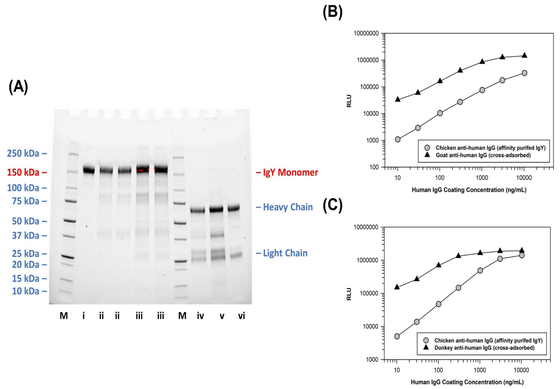
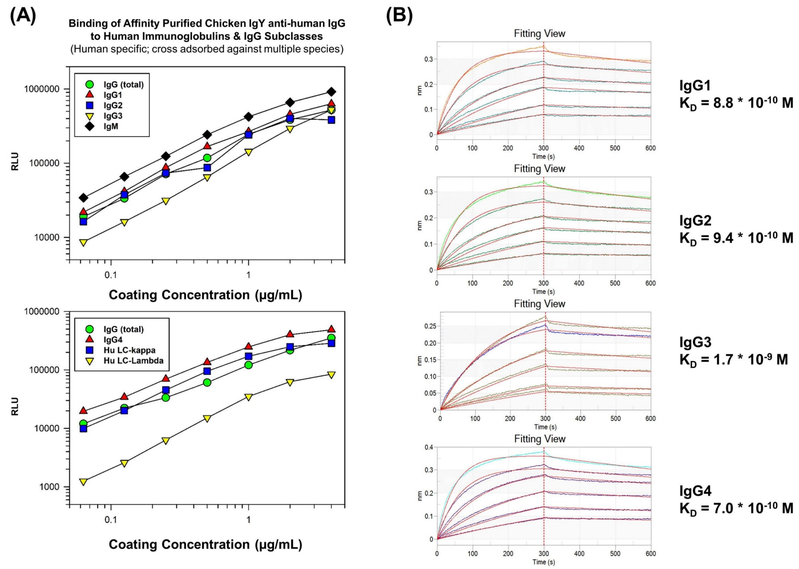


Figure 3. (A) Comparison of human IgG isotypes, human IgM, and human light chains by ECLIA method. Human IgG1, IgG2, and IgG4 all bind strongly to ruthenylated anti-human IgG chicken IgY. Human IgG3 binds somewhat less strongly. Human kappa light chain binds with similar strength to total IgG and IgG4 suggesting that the epitope for the chicken IgY may reside on the light chain. Lambda light chain binding was significantly weaker. (B) Biolayer interferometry (BLI) comparison of human isotype binding to biotinylated chicken IgY immobilized on streptavidin-functionalized biosensors. The trend observed in ECLIA is reproduced in BLI.



RESULTS

Unconjugated IgY indicated that the cross-reactivity against several species had been sufficiently depleted prior to conjugation (Figure 1). The antibody was subsequently biotinylated and ruthenylated to produce capture and detection reagents. Both conjugates were isolated in high purity (> 85%, Figure 2A) and demonstrated superior tolerance to endogenous IgG in monkey serum (Figure 2B & C).

Purified IgY and conjugates were observed to bind multiple classes and subclasses of human antibodies, it was therefore hypothesized that the antibody might target human light chain epitopes (Figure 3). MSD assays screened both light chain isoforms, kappa and lambda, all IgG subclasses, and IgM. Kappa bound more strongly than lambda, and comparable to IgG subclasses. This evidence supports that the IgY preferentially recognizes kappa light chains.

Jacobsen (2011) reported > 90% homology for human and monkey IgG subclass constant regions. Sequence differences were largely found in the hinge regions. Alignment of human and cynomolgus κ light chains showed stretches of homology punctuated by point mutations. It seems unlikely that cynomolgus serum preferentially depleted the lambda-specific antibodies from pooled IgY. More likely, κ immunoglobulins which are typically more abundant in human serum, are more immunogenic in chickens than λ immunoglobulins. Since the source immunogen was originally purified against protein A, and it is known that protein A does not bind IgG3 well, it is possible that no heavy-chain epitopes of IgG3 were recognized by the study animals and all reactivity against IgG3 results from light chain epitopes.

CONCLUSION(S)

We have prepared a chicken antibody which recognizes all human IgG subclasses. Binding strength was quantified using MSD and Sartorius Octet platforms. Results agreed across platforms, indicating interchangability. MSD assays incorporating monkey serum showed superior performance of IgY versus commercial antibodies. The chicken IgY appears to bind κ LC constant domain. IgG3 showed lowest affinity for any subclass, suggesting there are different affinities for hinge regions between subclasses. Remaining IgG subclasses showed K_D ~ 1 nM.

REFERENCES

1. Molecular and Functional Characterization of Cynomolgus Monkey IgG Subclasses. Frederick W. Jacobsen, Rupa Padaki, Arvia E. Morris, Teri L. Aldrich, Richard J. Armitage, Martin J. Allen, Jennifer C. Lavallee and Taruna Arora. J. Immunol. January 1, 2011, 186 (1) 341-349

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