Online Repository for IgG Subclass Distributions in Serum

By

Donald MacGlashan, Jr.

Santiago Alvarez-Arango

Jody Tversky

Johns Hopkins University

Johns Hopkins Asthma and Allergy Center

5501 Hopkins Bayview Circle

Baltimore, MD 21224
Subjects

Subjects were recruited under three Johns Hopkins University IRB-approved protocols for serum collection. Initial collections were quasi-random and atopic history was ascertained solely by historical account of a physician diagnosis of allergies from the subjects, based on skin prick tests and including whether they had obtained some form of allergen immunotherapy. For the subjects who do not report "allergies" in general or to the two allergens examined by ELISA, there was no corroborating skin test data. A secondary group was recruited from the Johns Hopkins Allergy clinic prior to or after they had obtained subcutaneous allergen immunotherapy. Once again, subject atopy was confirmed by skin prick test data.

Serum was obtained from blood samples after clotting and stored at -80°C.

ELISA construction

ELISA plates Nunc MaxiSorp flat bottom plates were coated overnight at room temperature with allergen solutions. Only mixed allergens were used. Lyophilized Short Ragweed (Greer Laboratories) was diluted to a concentration of 4 µg/ml in PBS and 0.3 ml loaded into each well of the plate. The plates were washed 5 times with PBS containing 0.02% Tween-20 and 0.1% BSA before adding samples or standards. Washing after overnight adsorption was followed by 10-15 minutes of blocking in washing buffer containing 0.1% BSA. Dermatophagoides farinae (Df) and Dermatophagoides pteronyssinus (Dp) from Allermed (10,000 BAU/ml stock in 50% glycerin) were coated as a mixture, each at 5 BAU/ml final concentration in PBS. Human serum albumin was coated at 2 µg/ml in PBS for the pilot experiments.
to determine nonspecific binding. Standards and serum samples were incubated in the plates for 1 hour at room temperature in PBS + 0.02% Tween-20 and 0.1% BSA. Typically 1/20 and 1/100 dilutions of the serum samples were added to the assay wells. After 4 washes with PBS/0.02% Tween-20, anti-subclass antibodies (anti-IgG1, HP6070P; anti-IgG2, HP6002P; anti-IgG3, HP6047P; anti-IgG4, HP6023P, diluted in PBS/0.02% Tween-20) were added at concentrations of 10 µg/ml, 4 µg/ml, 6 ng/ml and 1 µg/ml, respectively (see next section for calibration choices). After a one-hour incubation at room temperature and 4 washes, a 1/4000 dilution of anti-mouse IgG-HRP (Amersham, Inc.) was added for a one-hour incubation at room temperature. After 5 washes, TMB solution was added and the reaction stopped with 1M H₂SO₄.

In pilot experiments, nonspecific binding in this ELISA format (with serum present in the first incubation) was assessed by comparing binding in wells coated with HSA or coated with ragweed. For the IgG1, 2 and 4 subclasses, nonspecific binding to HSA was less than 1-5% and about 7% for IgG3. Sera were also adsorbed with sepharose beads coupled to either HSA or ragweed (CNBr-enabled coupling) and retested in the ELISA; approximately 90% of the signal was lost by adsorption with ragweed-beads and not with HSA-beads. In addition, dilution studies of test sera demonstrated that there was not an indication that one antibody subclass could block access by other subclasses, notably, that antigen-specific IgG1 was not overwhelming the ability of lower concentrations of IgG3 or IgG4 to be detected.

Cross-calibration of anti-subclass antibodies
The transfectoma series of subclass antibodies (IgG1, IgG2, IgG3, IgG4) [1] specific for a p-nitrophenyl ligand were used to cross-calibrate the anti-subclass antibodies. Pilot studies calibrated these transfectoma antibodies with a total IgG ELISA where the detection antibody was the WHO Ab 6045P [2]. Human IgG (ICN Laboratories) was used as the standard. With equal concentrations of the transfectoma subclass antibodies incubated on a plate, the anti-subclass antibodies were titrated to generate similar optical densities in the final reading. This balancing resulted in the concentrations of the anti-subclass antibodies used above (10 µg/ml, 4 µg/ml, 6 ng/ml, and 1 µg/ml for anti-IgG1, HP6070P; anti-IgG2, HP6002P; anti-IgG3, HP6047P; anti-IgG4, HP6023P, respectively).

For absolute calibration, on a single plate, one-third was loaded with ragweed (4 µg/ml in PBS), a third loaded with NP(7)-BSA (10 µg/ml in PBS) and a third loaded with equal mixture of D.P and D.F. dust mite allergens described above. For the ragweed portion, the serum standard was loaded at a range of concentrations while for the portion adsorbed to NP-BSA, anti-NP specific IgG1, IgG2, IgG3 or IgG4 was loaded at 15 ng/ml (for each subclass) and in the portion adsorbed to dust-mite, the chosen serum standard for dust-mite assays. After a one-hour incubation and washing, anti-subclass antibodies (see above) were incubated for 1 hour at room temperature. After washing, the wells were incubated with anti-mouse HRP antibody for 1 one-hour at room temperature. After washing, TMB solution was added and the color reaction stopped with 1M H$_2$SO$_4$. From the titration curves for a serum standard and subclass antibodies, equivalence (equal optical density) was calculated for the serum standard. These results allowed the absolute
concentrations of anti-ragweed subclass antibody to be determined and cross-calibrated the ragweed and dust mite standards for each subclass.

References

1. Bruggemann M, Williams GT, Bindon CI, et al. (1987) Comparison of the effector functions of human immunoglobulins using a matched set of chimeric antibodies. Journal of Experimental Medicine 166, 1351-61.

2. MacGlashan D, Jr., Hamilton RG. (2016) Parameters determining the efficacy of CD32 to inhibit activation of Fc epsilon RI in human basophils. J Allergy Clin Immunol 137, 1256-8 e1-11.