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Antigen nature and complexity influence human antibody light chain usage and specificity

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ABSTRACT

Human antibodies consist of a heavy chain and one of two possible light chains, kappa (κ) or lambda (λ). Here we tested how these two possible light chains influence the overall antibody response to polysaccharide and protein antigens by measuring light chain usage in human monoclonal antibodies from antibody secreting cells obtained following vaccination with Pneumovax23. Remarkably, we found that individuals displayed restricted light chain usage to certain serotypes and that lambda antibodies have different specificities and modes of cross-reactivity than kappa antibodies. Thus, at both the monoclonal (7 kappa, no lambda) and serum levels (145 μg/mL kappa, 2.82 μg/mL lambda), antibodies to cell wall polysaccharide were nearly always kappa. The pneumococcal reference serum 007sp was analyzed for light chain usage to 12 pneumococcal serotypes for which it is well characterized. Similar to results at the monoclonal level, certain serotypes tended to favor one of the light chains (14 and 19A, lambda; 6A and 23F, kappa). We also explored differences in light chain usage at the serum level to a variety of antigens. We examined serum antibodies to diphtheria toxin mutant CRM197 and Epstein-Barr virus protein EBNA-1. These responses tended to be kappa dominant (average kappa-to-lambda ratios of 4.52 and 9.72 respectively). Responses to the influenza vaccine were more balanced with kappa-to-lambda ratio averages having slight strain variations: seasonal H1N1, 1.1; H3N2, 0.96; B, 0.91. We conclude that antigens with limited epitopes tend to produce antibodies with restricted light chain usage and that in most individuals, antibodies with lambda light chains have specificities different and complementary to kappa-containing antibodies.

1. Introduction

Antibodies from humans and other mammals consist of a heavy chain (IgG, IgM, IgA, IgD, or IgE) and one of two possible light chains, kappa (κ) or lambda (λ). Both light chain gene products are similar in overall structure, consisting of a variable region (V), joining region (J), and constant region (C). Although slightly different in length (Vκ: ~107 aa V, 12 aa J and 107aa C region; Vλ: 95–98 aa V, 13 aa J and 105 aa C region) [1], both have the same structural motifs (complementarity determining-regions, etc.) and both similarly form disulfide bonds with the heavy chain. Human serum antibodies show a range in the ratio of kappa to lambda usage from 0.85 to 1.86 [2]. Swine are similar to humans with a close to 1:1 ratio, however rodents strongly favor kappa (20:1) [3] and horses, cows, dogs and cats strongly favor lambda [4].

Despite structural similarities at the protein level, the loci are quite different at the genetic level. The κ-light chain locus is on chromosome 2 while the λ-light chain locus is on chromosome 22. The kappa locus has only one possible constant region, whereas the lambda locus has seven, although only four are functional. Variable segments, however, are quite similar with roughly forty functional V genes from each light chain locus. Additional diversity, as with the heavy chain, comes from VJ junctions, P or N nucleotide additions, and somatic hypermutation.

B cells are capable of both salvaging faulty receptors and 'editing' their receptor to remove self-reactivity. In humans and mice, λ-light chain gene recombination only occurs after both kappa alleles fail to rearrange productively [5,6] or via receptor editing

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[7,8]. A B cell that switches to the lambda light chain requires additional B cell receptor signaling that is not needed for kappa editing [9]. Lambda light chain B cells can also arise by positive selection whereas secondary recombination can occur in the periphery, in a process known as receptor revision [10,11]. Thus, the existence of a significant percentage of serum lambda light chain antibodies is due to either failed kappa recombination or various stages of editing/revision during selection.

Preferential lambda antibody expression has been observed in a variety of situations in healthy individuals. Approximately 90% of IgD-secreting plasma cells have utilized the lambda chain [12]. Lambda chain usage was observed in other rare B cell populations [13]. Although various studies have examined the importance of the human serum kappa-to-lambda ratio for specific antigens in autoimmune disease [14–17] and allergy [18], to the best of our knowledge, no one has examined the serum kappa to lambda ratio in response to a variety of antigens in healthy individuals.

Our rationale was to examine light chain usage for antigen-specific serum and monoclonal antibodies as it may provide information into how B cells respond to different antigens and give us insight into structural motifs which may trigger certain antibody family usage. To this end, we examined kappa to lambda ratios that emerge in response to diphtheria toxin mutant CRM197, Epstein-Barr viral protein Epstein-Barr virus nuclear antigen-1 (EBNA-1), and to influenza and pneumococcal serotypes after vaccination with the trivalent flu vaccine (TIV) and Pneumovax23 respectively. Furthermore, we sorted, cloned, and expressed antibodies from single antibody secreting cells after vaccination with Pneumovax23. We show that for antibodies resulting from epitope-restricted (polysaccharide) vaccination, the lambda and kappa compartments show unique and distinct, yet complementary specificities.

2. Materials and methods

2.1. Human subjects

Serum lambda and kappa levels were measured from plasma obtained from two cohorts. The first cohort (Cohort 1) consisted of plasma samples from healthy donors from the Oklahoma Immune Cohort [19]. This cohort is well characterized for autoantibodies and all donors chosen for this study were autoantibody negative. The second cohort (Cohort 2) was plasma samples from healthy individuals obtained 6 weeks following vaccination with the 2008–2009 influenza vaccine (A/Brisbane/59/2007 (H1N1)-like virus, A/Brisbane/10/2007 (H3N2)-like virus and B/Florida/4/2006-like virus). All protocols were approved by the OMRF Institutional Review Board, and patients consented to participate.

Human monoclonal antibodies were prepared from one donor previously described (PVAX4, [20]), as well as two new donors PVAX5 and PVAX6. These donors received Pneumovax23 (Merck, Whitehouse Station, NJ) as standard of care vaccination based upon their age or diagnosis of systemic lupus erythematosus (SLE). Donor PVAX5 was a Caucasian male, age 63 with no known autoimmune disease. Donors PVAX4 and PVAX6 were Caucasian females with SLE, ages 45 and 38 respectively. Blood was drawn (~50 ml) into ACD vacutainers (BD, Franklin Lakes, NJ) by venipuncture seven days post vaccination and was stored no longer than 18 h before processing.

2.2. ELISA for measuring total kappa/lambda, anti-EBNA-1, anti-influenza and anti-CRM197 kappa/lambda IgG concentrations

High-binding plates (Costar 3369, Corning, Corning, NY) were coated with 100 μl/well of CRM197 (Reagent Proteins, San Diego, CA), EBNA-1 (Meridian Life Sciences, Memphis, TN) or Goat anti-human IgG (Bethyl Laboratories, Montgomery, TX) at a final concentration of 1 μg/mL in carbonate coating buffer. For influenza assays, plates were coated with 50 μl/well of egg-grown and sucrose gradient purified influenza strains A/Brisbane/59/2007, A/Uruguay/716/2007 and B/Florida/4/2006 at 16 HAU/well. Standard curves were generated using our own monoclonal IgKappa (PVAX6p1-D03) and IgLambda (PVAX6p8G02) antibodies, starting at 1 μg/mL in carbonate coating buffer followed by 2-fold serial dilutions, coated directly on the plate. Coated plates were allowed to incubate overnight at 4 °C. Plates were washed 5 times with PBS containing 0.05% Tween20. To block non-specific binding, 150 μl/well PBS with 20% FBS was added to plates coated with CRM197 and influenza or 150 μl/well PBS with 0.1% BSA was added to plates coated with EBNA-1 and anti-human IgG. Plates were incubated for 1 h at room temperature. After 5 washes, samples (100ul/well) were added in duplicate and plates were incubated at room temperature for 2 h. Samples were diluted in PBS with 2% FBS for CRM-197 and influenza and PBS only for EBNA-1 and anti-IgG ELISA's respectively. Samples were diluted 1:100 and 1:500 for CRM197 ELISAs and 1:128 followed by 3 serial 2-fold dilutions for influenza ELISAs. Samples were diluted 1:100 and 1:1000 for EBNA-1 ELISAs and 1:40,000 followed by 3 serial 2-fold dilutions for measuring total kappa/lambda concentrations. After washing, 100 μl/well of alkaline phosphatase conjugated anti-human IgKappa or anti-human IgLambda (Bethyl Laboratories, Montgomery, TX) diluted to 1:5000 in appropriate diluent (same as sample diluent) were added to the appropriate plates. Plates were incubated for 1 h at room temperature. To develop the plates, 100 μl/well of 1 mg/ml 4-nitrophenol phosphate (PNPP) (Sigma–Aldrich, Saint Louis, MO) in substrate buffer (1 M diethanolamine, 0.5 mM MgCl2) (Sigma–Aldrich, Saint Louis, MO) was added and after 15–20 min absorbances were measured at 405 nm. A reference wavelength of 490 nm was used in CRM197 ELISAs.

2.3. ELISA for measuring anti-serotype-specific polysaccharide kappa/lambda antibody concentrations

ELISA assays measuring antibody to *S. pneumoniae* polysaccharides were performed as per the WHO Gold Standard protocol [21] with modifications. Briefly, medium-binding plates (Costar 9017, Corning, Corning, NY) were coated with 100 μl/well of coating buffer (PBS/0.02% sodium azide) containing 2.5 μg/ml of cell wall polysaccharide (CWPS) (MiraVista Labs, Indianapolis, IN), serotype 9V and 14, 5 μg/mL of serotype 1, 4, 7F, 18C and 19A and 10 μg/ml of serotype 3, 5, 6A, 19F and 23F (all capsular polysaccharides from ATCC, Masassas, VA). Standard curves were generated by coating wells with serotype 4 polysaccharide and using monoclonal kappa (PVAX6p2G04) and lambda (PVAX5p4D05)-specific antibodies to serotype 4. Plates were covered and incubated at 37 °C for 5 h and then stored over night at 4 °C. Plates were allowed to come to room temperature while Pneumococcal US reference serum 007sp (from the USFDA) was pre-absorbed for 30 min at room temperature in absorption buffer (antibody buffer (PBS/0.02% sodium azide/0.05% Tween20) containing 5 μg/mL CWPS and 22F). Reference serum was diluted 1:100 followed by 6 serial 2-fold dilutions. Pre-absorption was excluded when measuring anti-CWPS antibody concentrations. After washing the plates in wash buffer (TBS/0.1% Brij), 50 μl/well of pre-absorbed reference serum was added to the plates in duplicate. Plates were covered and incubated at room temperature for 2 h. After washing, 100 μl/well of anti-human IgKappa or anti-human IgLambda (Bethyl Laboratories, Montgomery, TX) diluted to 1:5000 in antibody buffer was added to the plates and incubated at room temperature for 2 h. Plates were developed by adding 100 μl/well of 1 mg/ml of PNPP in substrate buffer. Plates

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