Published in final edited form as:

Vaccine. 2016 May 27; 34(25): 2813–2820. doi:10.1016/j.vaccine.2016.04.040.

Antigen Nature and Complexity Influence Human Antibody Light Chain Usage and Specificity

Kenneth Smith^{a,*}, Hemangi Shah^{a,b,*}, Jennifer J. Muther^a, Angie L. Duke^a, Kathleen Haley^a, and Judith A. James^{a,c}

^aOklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104, USA

^bPamlico BioPharma Inc., 865 Research Parkway, Suite 400, Oklahoma City, OK 73104, USA

^cOklahoma University Health Science Center, 1100 N. Lindsay, Oklahoma City, OK 73104, USA

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 $\mu\text{g/mL}$ kappa, 2.82 $\mu\text{g/mL}$ 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.

Corresponding Author: Kenneth Smith, PhD, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104, Phone: (405) 271-3275; Fax: (405) 271-7063, ; Email: Kenneth-Smith@omrf.org.

*These authors contributed equally to this work.

5. Conflict of Interest Statement

Hemangi Shah is an employee of Pamlico BioPharma, Inc.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

lambda light chain; kappa light chain; human monoclonal antibodies; anti-polysaccharide; light chain usage

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 (V κ : ~107 aa V, 12 aa J and 107aa C region; V λ : 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 [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 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 (~50ml) into ACD vacutainers (BD, Franklin Lakes, NJ) by venipuncture seven days post vaccination and was stored no longer than 18 hours 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 hour at room temperature. After 5

washes, samples (100µl/well) were added in duplicate and plates were incubated at room temperature for 2 hours. 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 1:40000 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 hour 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 mins 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 (1XPBS/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 hours 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 mins at room temperature in absorption buffer (antibody buffer (1X 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 (1X 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 hours. After washing, 100 µl/well of anti-human IgKappa and 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 hours. Plates were developed by adding 100 µl/well of 1 mg/ml of PNPP in substrate buffer. Plates were allowed to develop for about 20 mins and read at 405 nm with a reference wavelength of 650 nm.

2.4 Production and characterization of human monoclonal antibodies

Human monoclonal antibodies from single B cells purified from antibody secreting cells (ASCs) were generated as previously described [20, 22, 23]. To sort ASCs from donor PVAX5, a new antibody was added to the sorting scheme to enable kappa and lambda expressing B cells to be sorted into separate plates (anti-lambda-Pacific Blue, clone

MHL-38, Biolegend, San Diego, CA). Polysaccharide ELISAs were used to screen for binding and calculate antibody affinities (Kd) [20].

2.5 Data analysis

Serum antibody concentrations were obtained from optical density data using GraphPad Prism (version 6) using the standardized curve-fitting four parameter logistic method. Error bars represent standard deviations of at least three values. Monoclonal antibody variable region sequences were analyzed using the International Immunogenetics Information System (IMGT, Montpellier, France, <http://imgt.cines.fr/>) as well as with in-house software. Clonally related antibodies were defined as those having the same VDJ/VJ usage in the heavy and light chains, and highly related V_HD_H , D_HJ_H , and V_KJ_K or V_LJ_L junctions.

3. Results

3.1 Kappa usage is enriched in diphtheria toxin serum antibodies

Before examining various kappa-to-lambda ratios in antibodies directed against several select antigens, the total IgG kappa-to-lambda ratio in individuals from Cohort 1 and Cohort 2 were determined (Supplemental Figure 1A). The ratio for cohort 1 fell mostly within the previously reported 0.85 to 1.86 range [2] (mean ratio: 1.27, median: 1.28, range: 0.783 to 1.59). Several individuals from Cohort 2 had slightly higher ratios (mean ratio: 1.66, median: 1.47, range: 1.34 to 2.79). The only notable difference between these groups is that Cohort 2 individuals were all recently vaccinated (samples drawn 6 weeks after influenza vaccination). Similarly, we determined the kappa-to-lambda ratio for IgG subclasses IgG₁, IgG₂, and IgG₄ for each individual in Cohort 1 (Supplemental Figure 1B). Although there were variations per individual, the average kappa-to-lambda ratios were similar to each other and the total ratios determined above indicating no kappa or lambda preference for individual IgG subclasses (mean ratio IgG₁: 1.18, IgG₂: 1.26, IgG₄: 1.26).

We examined kappa-to-lambda ratios in antibodies that arise in twenty-eight healthy individuals from Cohort 1 and ten individuals from Cohort 2 following vaccination by the inactive diphtheria toxin mutant CRM197, a well-known protein antigen against which most of the U.S. population is well-vaccinated. Kappa and lambda antibody responses were measured and ratios calculated (Figure 1). We found the kappa-to-lambda ratios of most individuals were close to the 3:1 ratio (Figure 1, dashed line) (mean ratio: 4.52, median: 2.56, range: 1.09 to 29.9), with the exception of two individuals with extremely high kappa-utilizing antibody responses.

We included in these measurements the “new” pneumococcal reference serum, 007sp. This serum is pooled from 225 healthy adults who were given Pneumovax23 and thus the ratio represents an average kappa-to-lambda ratio for 225 individuals. It is likely that these individuals were also properly vaccinated against diphtheria toxin and therefore the 007sp ratio of 5.23 is an important standard measure. Overall, we found the anti-CRM response was skewed toward kappa antibodies.

3.2 Balanced kappa-to-lambda ratios against influenza

We examined the kappa-to-lambda usage ratio in antibodies isolated from influenza vaccinated individuals (Cohort 2) using plasma collected 6 weeks following vaccination with the '08-'09 vaccine (H3N2, A.Uruguay; H1N1, A.Brisbane; Shanghai lineage, B.Florida) (Figure 2). The trivalent influenza vaccine consists of a standardized amount of HA and possibly smaller amounts of other influenza proteins including neuraminidase (NA). The kappa-to-lambda usage ratios in antibodies that react to each of the strains in the vaccine were H1N1, mean: 1.07, median: 1.16, range: 0.507 to 1.38; H3N2, mean: 0.956, median: 1.02, range: 0.539 to 1.29; and B strain, mean: 0.908, median: 0.833, range: 0.478 to 1.58, showing that the B strain antibodies tended to be somewhat lambda dominant. With the exception of one individual (labeled 590038) who was lambda dominant for all three strains, the kappa-to-lambda usage ratio fluctuated for each donor depending on the strain that the antibodies bound.

3.3 The kappa-to-lambda ratio against EBNA-1 is strongly kappa skewed

We next compared kappa to lambda ratios in antibodies to EBNA-1, an antigen that is known to be kappa skewed [15]. EBNA-1 is the major latent protein of the EBV virus and is responsible for maintaining the episomal state of EBV DNA in infected cells. Over 90% of adults have antibodies against EBNA-1 [24], the majority of which are directed against a glycine-alanine repeat domain, a highly antigenic portion of the protein [25], inhibiting effective responses to eliminate the latent virus. Although the pneumococcal reference serum shows a 5.2 kappa-to-lambda ratio (Figure 3), the kappa-to-lambda ratios in anti-EBNA-1 of some individuals in our cohorts were as high as 25.7 (subject 541016) with a mean ratio of 9.72, median: 7.61, range: 0.627 to 25.7.

3.4 Kappa to lambda ratios for anti-polysaccharide are serotype dependent

We assayed the pneumococcal reference serum 007sp on 12 well-characterized pneumococcal serotypes using the World Health Organizations' "gold" standard ELISA for kappa-to-lambda antibody usage ratios. Across all 12 serotypes assayed the average ratio was 1.88. Interestingly, unlike the protein antigens assayed above, some serotypes were lambda dominant. Serotypes 14 and 19A both had kappa-to-lambda ratios less than 1 (0.70 and 0.73) (Figure 4A). Conversely, the highest kappa-to-lambda ratio was for serotypes 6A and 23F at 3.2 and 3.5, respectively. Serotypes 3, 5, 7F, and 9V were just above 1:1 (1.2, 1.5, 1.2, 1.5 respectively).

We also examined the kappa-to-lambda ratio for antibodies directed against *Streptococcus pneumoniae* cell wall polysaccharide (CWPS) in the reference serum. CWPS is a known contaminant in the capsular polysaccharides and elicits an immune response similar to the capsular polysaccharides [20]. Anti-CWPS is typically not an opsonin and is "absorbed" from samples before analyzing the antibody response by ELISA according to WHO standards. To measure anti-CWPS, we did not pre-absorb the reference serum and quantified kappa and lambda on plates coated with purified CWPS. Remarkably, nearly all of the anti-CWPS present in the reference serum was kappa (Figure 4B, 145 µg/ml kappa, 2.82 µg/ml lambda; >50:1 ratio).

3.5 Human monoclonal antibodies to polysaccharide have differing specificities based on their light chain usage

To more definitively determine how kappa and lambda light chains contribute to the human antibody response to Pneumovax23, we made monoclonal antibodies from single ASCs. In a previous report, our lab produced and characterized 150 fully human, full-length monoclonal anti-polysaccharides [20]. All of those antibodies utilized the kappa light chain due to experimental constraints. For this study, lambda antibodies were cloned from one donor from the previous report (PVAX4) and another donor was processed for both lambda and kappa antibodies (PVAX6). For PVAX5, an anti-lambda was added to the sorting strategy so that individual lambda and kappa B cells could be easily isolated.

Figure 5 shows specificities from each of the three donors, highlighted for either utilizing kappa or lambda light chain. Some serotypes, especially those with a large overall response (remembering that all ASCs and their resulting antibodies arise from an anamnestic response), used both kappa and lambda (PVAX4, serotype 5; PVAX5, serotype 33F; PVAX6, serotype 9V). However, for the most part, the monoclonal antibodies generated to different serotypes used *either* kappa or lambda light chains. For example, PVAX5 used only kappa for serotypes 1, 11A, 12F, 14, 15B, 17F, 19F, and CWPS, and only lambda for 6B, 8, 9N, 19A, and 22F. Antibodies from donors who made both kappa and lambda light chain-containing antibodies to a specific serotype had no general differences in affinity based on the light chain used (data not shown).

We showed previously [20] that as much as 20% of the ASC response to Pneumovax23 is dominated by antibody to CWPS which does not contribute to overall opsonic activity. Like the serum antibodies results (see above) we did not find a single lambda anti-CWPS; all antibodies utilized the kappa chain.

3.6 Human kappa and lambda containing monoclonal antibodies to polysaccharide exhibit different patterns of cross-reactivity

We previously showed [20] that 15% of the kappa-containing monoclonal antibodies we characterized had cross-reactivity to two serotypes, but never more than two. We found antibodies showing cross-reactivity to related serotypes 9N and 9V, and to related serotypes 19A and 19F; and even to less similar serotypes 14 and 15B and also 17F and 33F. Antibodies with lambda light chains demonstrated different cross-reactivities and none were the same as the kappa containing antibodies. Figure 6 shows binding curves for four such antibodies. PVAX4-p7B03 and PVAX5-p4A05 bind to 19A, but rather than also binding to 19F, they bind to 9V (Figure 6A) and not to 9N. PVAX5-p4A05 is also our first example of a high affinity 19A antibody (Kd: 0.17 nM). All of the antibody examples having kappa chain and binding to 19A and 19F antigens were of moderate to low affinity to both (Kd: <1.1 nM). Figure 6B shows PVAX5-p3E04 which binds to 19A as well, but cross-reacts with 6B. Finally, Figure 6C shows an example of an antibody which binds significantly to four serotypes. PVAX5-p4G04 binds with high affinity to 9N and 9V (Kd: 0.28 nM), which was seen with some kappa-bearing antibodies, but also binds appreciably to 19A and 22F, but not 19F. It is notable that lambda anti-polysaccharides represent an entire compartment of different specificities and cross-reactivities.

4. Discussion

While it is well known in humans (and mice) that lambda light chains are important in the overall immune response. No study, to the best of our knowledge, has used modern ELISA and monoclonal antibody techniques to dissect the difference in kappa and lambda usage in a variety of antigen-specific humoral responses. A recent version of Janeway's "Immunobiology" [26] textbook states that "No functional difference has been found between antibodies having λ or light chains...". Indeed, in mice, there was little difference between the kinetics of binding to the dansyl hapten for antibodies with kappa or lambda light chains [27]. However, light chain usage is clearly influenced by the nature of the antigen, and as we show here, the light chain clearly influences specificity, or likely vice versa.

We used the mutant diphtheria toxin protein CRM197 to explore kappa-to-lambda ratios, representing an antigen against which nearly all humans are well vaccinated. CRM197 differs from diphtheria toxin by a single amino acid mutation and is used as a protein conjugate in polysaccharide conjugate vaccines (i.e. Prevnar13) to elicit a T-dependent response. The diphtheria toxin antigen, a moderate-sized protein (58.4 kDa), induces a kappa-dominant antibody response. This situation contrasts with influenza HA, a large, highly glycosylated protein that is experienced by the immune system as complex multimers which differ from virus to virus (and year to year) by mutations in the particularly antigenic regions. Our results show that diphtheria toxin antibody responses had kappa-to-lambda ratios of 3:1 and influenza responses were nearer to 1:1. These results suggest that memory B cells utilizing both the kappa and lambda light chains are important to the immune response against HA, but the response to the smaller, 'simpler' antigen CRM197 is sufficiently diverse with predominant usage of one light chain, in this case kappa.

Based on these data, we thought that smaller antigens might have limited epitopes and thus fewer potential naïve B cells that can recognize those epitopes. The smaller antigens might then elicit an antibody response preferring one particular light chain. This hypothesis was confirmed in the antibody response to EBNA-1, where most antibodies in healthy individuals are directed to the glycine-alanine repeat region [15]. We found that antibody responses to EBNA-1 as a whole are strongly kappa dominant in healthy individuals where kappa-utilizing antibodies outnumbered lambda-utilizing antibodies by nearly 10 to 1. This virus has been studied in detail, in part due to its link to systemic lupus erythematosus [28, 29]. In one particular study [15] it was found that healthy individuals mounted an immune response to the GA repeat which was highly kappa skewed, whereas SLE patients had a more diverse response against EBNA-1 and had equal amounts of serum kappa and lambda anti-EBNA-1. Thus, even in the same antigen, the 'simple' epitope induces a kappa-dominant response, whereas the rest of the protein induces a more balanced response.

A better example of responses to 'epitope-limited' antigens is the response to *Streptococcus pneumoniae* CWPS. Previous work [30] showed that the immune repertoire to *Haemophilus influenza* type b capsular polysaccharide is usually kappa dominated. However, certain individuals exclusively express antibodies using the lambda light chain, and these antibodies are often dominated by particular lambda variable regions. More recently, our lab has shown

that most antibodies against CWPS, even from different donors, use the same V-family (VH3-30/VH3-33) and have remarkably similar CDR3s [20]. We have also shown here that nearly all of the anti-CWPS humoral response utilizes the kappa chain, both at the monoclonal and plasma levels. Similarly, certain polysaccharides (as shown with the 007sp reference serum), favor one light chain or the other, and in any particular individual, responses to certain polysaccharides, even at the monoclonal level, favor one light chain or the other. Together, these data are consistent with our hypothesis that ‘epitope-limited’ antigens have limited repertoires in the human immune system.

It is remarkable that the lambda light chain has been retained evolutionarily as an important component of the immune response in humans. Multiple failed kappa rearrangements are necessary before lambda usage, and yet lambda-bearing antibodies are roughly half the overall serum antibody. These findings imply that lambda chains are necessary for proper antigen-specific immune repertoires and diversity. Our analysis of monoclonal anti-polysaccharides demonstrates that different donors show clear lambda or kappa preferences to various serotypes (Figure 5A–C). Together, these data indicate that the lambda chain acts as a second compartment of specificities to broaden the repertoire, and its usage is different and complementary to the kappa response. It has also recently been suggested that lambda chains have a more varied ‘structural repertoire’ than kappa [31]. Generation of antibodies with lambda light chain after multiple kappa failures may result in a more effective antibody against a particular antigen.

Because all of the monoclonal antibodies we examined here are anti-polysaccharide, and the majority of them use the IgG₂ subclass, we were also interested whether different IgG subclasses tend to have different kappa-to-lambda ratios. Of all of the monoclonal antibodies from our anti-polysaccharide library (191 antibodies), 90.5% were the IgG₂ subclass. Curiously, the 18 antibodies that bound polysaccharide and used the IgG1 subclass were all kappa light chain, but 15 of these were from two of the four donors. This again indicates that kappa to lambda preferences are specific to the individual. Furthermore, we also examined this at the serum level (Supplemental Figure 1B). For each donor in cohort 1, we examined the kappa-to-lambda ratio for IgG₁, IgG₂, and IgG₄. As shown for total IgG kappa-to-lambda ratios, each donor has different distributions of kappa and lambda, but when all eight donors were averaged per subclass, the averages are similar, with little difference between IgG₁, IgG₂, and IgG₄. We are further examining whether this is true for IgM and IgA.

It is curious that different individuals show kappa or lambda preference to certain immunogens. In mice, genetic background has a significant effect on immune response. T-independent antigens (NP-Ficoll) generated over 40% lambda antibodies in contrast to the normal 5% usage. However, the kappa-to-lambda ratio for a T-dependent antigen (NP-OVA), varied depending on the mouse strain (DBA/2 and BALB/c: 40–50%, NZB and C3H: <10%) [32]. To our knowledge, this issue has not been explored in humans. In our small sample set and small number of antigens explored, no individual was found with predominant usage of either kappa or lambda across all of the antigens we analyzed. For example, donor 500224 had a relatively high total kappa to lambda ratio, but was near the average for CRM197, EBNA-1 and flu. Thus, in our small data set, we found no general predisposition for certain healthy individuals to use one light chain over the other.

Perhaps upon first exposure to antigen, activation of naïve cells in the germinal center depends on competition for, and acquisition of scarce antigen, followed by B cell stimulation and survival signals. If so, an advantage might exist for the B cell that arrives first, regardless of the light chain it possesses. If kappa and lambda repertoires can both bind an antigen, one individual might end up with a kappa response to that antigen, but another may be lambda. Nevertheless, certain antigens are better bound by either kappa or lambda repertoires in almost all individuals (i.e., CWPS or 7F). In our own studies, we were unable to find antibodies to polysaccharide 7F until extending our molecular biology techniques to include the lambda light chain. The lambda light chain also supported high affinity anti-19A with no cross-reactivity to 19F, whereas the kappa light chain only produced low affinity anti-19A that also cross-reacted with 19F.

Overall, our results show that immunization with certain antigens typically induces antibodies favoring one light chain or the other. This is especially true with antigens that are ‘epitope limited’. Our findings reveal the importance of producing and characterizing antibodies with both light chain families when analyzing a humoral response at the monoclonal level or when looking for novel antibody specificities. Most importantly, we show that specificities in antibodies with lambda light chains are different and complementary to those with kappa light chains. Although lambda usage results from failure, it is crucial to the success of the immune response.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank J. Donald Capra for many helpful discussions about this work, we miss you immensely. We also thank our donors for this study, as well as our clinical staff Virginia Roberts and Jeremy Levin. We thank Lori Garman, Linda Thompson and Mark Coggeshall for editing the manuscript. We also thank Emily McKee, Jennifer VanDeventer, Jacob Bass, and Diana Hamilton for technical assistance. Finally, purified influenza virus was a kind gift from Gillian Air and reference serum 007sp from Dr. Mustafa Akkoyunlu at the US FDA. This research has been supported by National Institutes of Health Grants P30GM103510, P30AR053483, U19AI062629, U19AI082714, U54GM104938, U01AI01934 and contract HHSN266200500026C, as well as Pamlico BioPharma, Inc.

References

1. Solomon A, Weiss DT. Structural and functional properties of human λ -light-chain variable-region subgroups. *Clin Diag Lab Immunol*. 1995; 2:387–94.
2. Barandun S, Morell A, Skvaril F, Oberdorfer A. Deficiency of kappa- or lambda-type immunoglobulins. *Blood*. 1976; 47:79–89. [PubMed: 812574]
3. Woloschak GE, Krco CJ. Regulation of kappa/lambda immunoglobulin light chain expression in normal murine lymphocytes. *Mol Immunol*. 1987; 24:751–57. [PubMed: 3116408]
4. Arun SS, Breuer W, Hermanns W. Immunohistochemical examination of light chain expression (lambda/kappa ratio) in canine, feline, equine, bovine and porcine plasma cells. *Zentralbl Veterinarmed A*. 1996; 43:573–76. [PubMed: 8968166]
5. Durdik J, Moore MW, Selsing E. Novel kappa light-chain gene rearrangements in mouse lambda light chain-producing B lymphocytes. *Nature*. 1984; 307:749–52. [PubMed: 6422305]

6. van der Berg M, Tumkaya T, Boerma M, de Bruin-Versteeg S, Langerak AW, van Dongen JJ. Ordered recombination of immunoglobulin light chain genes occurs at the IGK locus but seems less strict at the IGL locus. *Blood*. 2001; 97:1001–8. [PubMed: 11159529]
7. Gay D, Saunders T, Camper S, Weigert M. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med*. 1993; 177:999–8. [PubMed: 8459227]
8. Tiegs SL, Russell DM, Nemazee D. Receptor editing in self-reactive bone marrow B cells. *J Exp Med*. 1993; 177:1009–20. [PubMed: 8459201]
9. Derudder E, Cadera EJ, Vahl JC, Wang J, Fox CJ, Zha S, et al. Development of immunoglobulin λ -chain-positive B cells, but not editing of immunoglobulin κ -chain, depends on NF- κ B signals. *Nat Immunol*. 2009; 10:647–54. [PubMed: 19412180]
10. Meffre E, Papavasiliou F, Cohen P, de Bouteiller P, Bell D, Karasuyama H, et al. Antigen receptor engagement turns off the V(D)J recombination machinery in human tonsil B cells. *J Exp Med*. 1998; 188:765–72. [PubMed: 9705958]
11. Wilson PC, Wilson K, Liu YJ, Banchereau J, Pascual V, Capra JD. Receptor revision of immunoglobulin heavy chain variable region genes in normal human B lymphocytes. *J Exp Med*. 2000; 191:1881–94. [PubMed: 10839804]
12. Arpin C, de Bouteiller O, Razanajaona D, Fugier-Vivier I, Briere F, Banchereau J, et al. The normal counterpart of IgD myeloma cells in germinal center displays extensively mutated IgVH gene, C μ -C δ switch, and λ light chain expression. *J Exp Med*. 1998; 187:1169–78. [PubMed: 9547329]
13. Duty JA, Szodoray P, Zheng NY, Koelsch KA, Zhang Q, Swiatowski M, et al. Functional anergy in a subpopulation of naïve B cells from healthy humans that express autoreactive immunoglobulin receptors. *J Exp Med*. 2009; 206:139–51. [PubMed: 19103878]
14. Riesen W, Keller H, Skvaril F, Morell A, Barandun S. Restriction of immunoglobulin heterogeneity, autoimmunity and serum protein levels in aged people. *Clin Exp Immunol*. 1976; 26:280–5. [PubMed: 62632]
15. Petersen J, Rhodes R, Roudier J, Vaughan JH. Altered immune response to glycine-rich sequences of Epstein-barr nuclear antigen-1 in patients with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum*. 1990; 33:993–1000. [PubMed: 2164400]
16. Schoettler N, Ni D, Weigert M. B cell receptor light chain repertoires show signs of selection with differences between groups of healthy individuals and SLE patients. *Mol Immunol*. 2012; 51:273–82. [PubMed: 22516082]
17. Nei T, Urano S, Itoh Y, Kitamura N, Hashimoto A, Tanaka T, et al. Light chain (κ/λ) ratio of GM-CSF autoantibodies is associated with disease severity in autoimmune pulmonary alveolar proteinosis. *Clin Immunol*. 2013; 149:357–64. [PubMed: 24211842]
18. Gadermaier E, Staikuniene J, Scheiblhofer S, Thalhamer J, Kundi M, Westritschnig K, et al. Recombinant allergen-based monitoring of antibody responses during injection grass pollen immunotherapy and after 5 years of discontinuation. *Allergy*. 2011; 66:1174–82. [PubMed: 21480924]
19. Ritterhouse LL, Lu R, Shah HB, Robertson JM, Fife DA, Maecker HT, et al. Vitamin D deficiency in a multiethnic healthy control cohort and altered immune response in vitamin D deficient European-american healthy controls. *PLoS One*. 2014; 9:e94500. [PubMed: 24727903]
20. Smith K, Muther J, Duke AL, McKee E, Zheng N, Wilson PC, et al. Fully human monoclonal antibodies from antibody secreting cells after vaccination with Pneumovax23 are serotype specific and facilitate opsonophagocytosis. *Immunobiology*. 2013; 218:745–54. [PubMed: 23084371]
21. Wernette CM, Frasca CE, Madore D, Carlone G, Goldblatt D, Plikaytis B, et al. Enzyme-linked immunosorbent assay for quantitation for human antibodies to pneumococcal polysaccharides. *Clin Vaccine Immunol*. 2003; 10:514–19.
22. Smith K, Garman L, Wrammert J, Zheng N, Capra JD, Ahmed R, et al. Rapid generation of fully human monoclonal antibodies specific to a vaccinating antigen. *Nat Prot*. 2009; 4:372–84.
23. Smith K, Crowe S, Garman L, Guthridge C, Muther J, McKee E, et al. Human monoclonal antibodies generated following vaccination with AVA provide neutralization by blocking furin cleavage but not by preventing oligomerization. *Vaccine*. 2012; 30:4267–83. [PubMed: 22521287]

24. De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. *World J Virol.* 2012; 1:31–43. [PubMed: 24175209]
25. Rumpold H, Rhodes GH, Bloch PL, Carson DA, Vaughan JH. The glycine-alanine repeating region is the major epitope of the Epstein-Barr nuclear antigen-1 (EBNA-1). *J Immunol.* 1987; 138:593–9. [PubMed: 2432130]
26. Janeway, C. *Immunobiology.* 7. New York: Garland; 2008.
27. Montano RF, Morrison SL. Influence of the isotype of the light chain on the properties of IgG. *J Immunol.* 2002; 168:224–31. [PubMed: 11751966]
28. McClain MT, Poole BD, Bruner BF, Kaufman KM, Harley JB, James JA. An altered immune response to Epstein-Barr nuclear antigen 1 in pediatric systemic lupus erythematosus. *Arthritis Rheum.* 2006; 54:360–68. [PubMed: 16385527]
29. Poole BD, Gross T, Maier S, Harley JB, James JA. Lupus-like autoantibody development in rabbits and mice after immunization with EBNA-1 fragments. *J Autoimmun.* 2008; 31:362–71. [PubMed: 18849143]
30. Adderson EE, Shackelford PG, Insel RA, Quinn A, Wilson PM, Carroll WL. Immunoglobulin light chain variable region gene sequences for human antibodies to *Haemophilus influenzae* type b capsular polysaccharide are dominated by a limited number of V κ and V λ segments and VJ combinations. *J Clin Invest.* 1992; 89:729–38. [PubMed: 1541667]
31. Chailyan A, Marcatili P, Cirillo D, Tramontano A. Structural repertoire of immunoglobulin λ light chains. *Proteins.* 2011; 79:1513–24. [PubMed: 21365679]
32. Ju S, Dorf M. Preferential induction of specific λ -isotypic antibodies in mice. *J Immunol.* 1984; 133:1404–9. [PubMed: 6430998]

Figure 1. Diphtheria toxin mutant CRM197 antibodies are enriched for kappa

Kappa-to-lambda ratios for twenty-eight individuals from cohort 1, ten individuals from cohort 2, and 007sp reference serum were determined for CRM197. The dotted line indicates a ratio of 3:1.

Figure 2. Influenza antibodies have a balanced kappa-to-lambda ratio

Kappa-to-lambda ratios were determined for ten individuals from plasma collected six weeks after influenza vaccination. The dotted line indicates a ratio of 1:1. Average ratios for influenza A were close to 1:1, influenza B slightly below.

Figure 3. Antibody responses to EBNA-1 are kappa skewed

Kappa-to-lambda ratios for thirteen individuals from cohort 1 and ten from cohort 2 were determined against full-length recombinant EBNA-1. The dotted line indicates a ratio of 3:1.

Figure 4. Kappa-to-lambda ratios for anti-polysaccharide are dependent on serotype

A) Kappa-to-lambda ratios against twelve pneumococcal serotypes were determined for 007sp reference serum. The dotted line indicates a ratio of 1:1. B) Total kappa and lambda anti-cell wall polysaccharide was determined for 007sp.

Figure 5. Anti-polysaccharide monoclonals favor kappa or lambda based on serotype and donor
 Histogram of characterized antibodies from three donors A) PVAX4, B) PVAX5, and C) PVAX6, binned by serotype. Kappa antibodies are colored black, lambda antibodies are gray. Arrows point to serotypes only bound by antibodies with either kappa (black) or lambda (gray) light chains.

Figure 6. Monoclonal antibodies with a lambda light chain that bind to more than one polysaccharide serotype

A) Two antibodies (PVAX4-p7B03 and PVAX6-p4A05) from two different donors which cross-react between 19A and 9V (but not 19F). B) Antibody PVAX6-p3E04 cross-reacts between 19A and 6B (but not 19F). C) Antibody PVAX6-p4G04 is the first antibody out of over 150 characterized that binds to more than two serotypes (19A, 9N, 9V and 22F).