Human B-cell isotype switching origins of IgE

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Background: B cells expressing IgE contribute to immunity against parasites and venoms and are the source of antigen specificity in allergic patients, yet the developmental pathways producing these B cells in human subjects remain a subject of debate. Much of our knowledge of IgE lineage development derives from model studies in mice rather than from human subjects.

Objective: We evaluate models for isotype switching to IgE in human subjects using immunoglobulin heavy chain (IGH) mutational lineage data.

Methods: We analyzed IGH repertoires in 9 allergic and 24 healthy adults using high-throughput DNA sequencing of 15,843,270 IGH rearrangements to identify clonal lineages of B cells containing members expressing IgE. Somatic mutations in IGH inherited from common ancestors within the clonal lineage are used to infer the relationships between B cells.

Results: Data from 613,641 multi-isotype B-cell clonal lineages, of which 592 include an IgE member, are consistent with indirect switching to IgE from IgG- or IgA-expressing lineage members in human subjects. We also find that these inferred isotype switching frequencies are similar in healthy and allergic subjects.

Conclusions: We found evidence that secondary isotype switching of mutated IgG_1-expressing B cells is the primary source of IgE in human subjects, with lesser contributions from precursors expressing other switched isotypes and rarely IgM or IgD, suggesting that IgE is derived from previously antigen-experienced B cells rather than naive B cells that typically express low-affinity unmutated antibodies. These data provide a basis from which to evaluate allergen-specific human antibody repertoires in healthy and diseased subjects. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *IgE, isotype switching, direct, indirect, antibody, B cell, repertoire, high-throughput DNA sequencing*

B cells producing antibodies of distinct isotypes are the basis for humoral immune defense but are also the source of pathogenic antibodies, such as allergen-specific IgE, in allergic subjects. Although mature but antigen-naive cells exclusively express IgM and IgD, antigen stimulation and an appropriate microenvironment of interaction with T cells and cytokines can trigger B-cell genomic rearrangements, leading to expression of IgG, IgA, or IgE isotype subclasses. Each of these subclasses has characteristic immune effector and modulatory functions, including activating the complement pathway, opsonizing antigens, binding to distinct Fc receptors, and in the case of IgE, providing antigen specificity for mast cells and basophil responses.[1,2]

In human subjects debate continues over the extent to which IgE isotype switching occurs directly from IgM or indirectly from other intermediate antibody isotypes, and this question has not been resolved, even in tractable model organisms, such as the mouse.[3-7] Recent studies with IgE reporter mice have been interpreted to support predominantly direct or indirect switching pathways to IgE.[8-13] For example, He et al[9] report evidence that IgE-positive germinal center cells in mice derive from direct switching, whereas IgE-positive plasma cells derive from sequential switching. Using a different reporter model, Talay et al[10,11] report that serum IgE in recall responses can be derived from IgE-positive memory cells and IgG_1-positive memory cells, presumably by differentiation to plasma cells in the first case and sequential isotype switching and plasma cell differentiation in the latter case. Yang et al,[12] using a third reporter mouse model, do not specifically address whether IgE derives from direct or indirect switching but find IgE-positive plasma cells with low mutation levels early in the immune response when IgE-positive germinal center B cells show increased mutation levels, suggesting that these IgE-positive cell types arise from different pathways.

Studies in human subjects, as reviewed by Davies et al,[14] suggest that direct switching from IgM to IgE production occurs in incompletely organized germinal centers and results in minimally mutated IgE exhibiting low affinity for antigen. In contrast, indirect switching to IgE production through other isotype intermediates is expected to result in IgE possessing a higher level of hypermutation

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Supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIAID) grants 1U19AI0420901 (S.D.B., PD) and U19AI090019 (M.M.D., PI; S.D.B., PD) and NIH/National Center for Research Resources CTSA award UL1 RR025744.

Disclosure of potential conflict of interest: C. L. Dekker has received research support from the National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NIAID; U19A1057229 [M. M. Davis] and U19AI090019 [M. M. Davis]), EMMES (NIH/VRC prime; VRC 703 [seasonal flu DNA vaccine]) and has received consultancy fees from Pfizer. M. M. Davis has received research support, payment for writing/reviewing the manuscript, and Provision of writing assistance, medicines, equipment, or administrative support from the NIH (U19 AI090019). S. D. Boyd has received research support from NIH/NIAID, Department of Defense, and Ellison Medical Foundation; has received consultancy fees from ImmuMetrix; has received lecture fees from the Clinical Immunology Society, the JASON program, the International Society of Laboratory Hematology, the Banff International Research Station, Keystone Symposia, Cold Spring Harbor Laboratories, Brigham Young University, the University of Nebraska, the XXI Mexican Congress of Immunology, the Hudson Alpha Institute, the University of Cincinnati, and the Illumina Scientific Advisory Board; has a US-TPO patent related to DNA sequence interpretation; and has stock in CareDx and Lineage Biosciences. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 5, 2015; revised June 29, 2015; accepted for publication July 1, 2015.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.07.014

Abbreviations used
CDR3: Complementarity determining region 3
FR: Framework region
IGH: Immunoglobulin heavy chain gene

and allergen affinity and would be accompanied by shared patterns of hypermutation among B cells expressing IgE and members of the same clonal lineage expressing other isotype intermediates. Importantly, class-switching is a 1-directional process because the genomic DNA between the upstream isotype and downstream isotype is excised during switching.

To obtain human data that could distinguish between the direct and indirect models of human B-cell switching to IgE and assess the extent to which direct and indirect switching are observed in healthy or allergic subjects, we performed deep sequencing of immunoglobulin heavy chain (IGH) cDNA derived from peripheral blood B cells taken from 24 healthy subjects and 9 patients with reported allergy to aeroallergens or food allergens. We identified clonal lineages containing IgE-expressing B cells, as well as lineage members expressing other immunoglobulin isotypes. Our data analysis takes advantage of the fact that antigen-stimulated B cells produce somatic mutations in their IGH V(D)J gene rearrangements at high rates, and these mutations are inherited by daughter cells, permitting reconstruction of relationships within clonal lineages based on the presence of shared mutations between different cells derived from a common ancestor. The results support a model for human IgE B-cell development, where isotype switching through intermediate isotypes is most common. In particular, we find that in both healthy and allergic subjects, IgE-encoding transcripts are most closely related in clonal lineages to IgG-encoding transcripts, particularly IgG_1, and share extensive patterns of hypermutation with this isotype, with a smaller fraction appearing to switch directly from B cells expressing IgM or IgD. The data suggest that the pathogenic IgE antibodies in allergic patients might differ from those in healthy subjects for reasons related to their antigenic specificities, affinities, or levels, rather than from major differences in the pathway of switching to IgE.

METHODS

Collection of clinical samples from human subjects

Peripheral blood samples were obtained from the Stanford Ellison influenza vaccination cohort, consisting of 19 healthy subjects (8 male and 11 female subjects; age range, 21-88 years) and 8 allergic patients (2 male and 7 female patients; age range, 24-84 years) and including longitudinal samples from 3 time points from each of 2 consecutive years collected as part of an ongoing vaccination study. Peripheral blood samples from an additional 5 healthy subjects (4 male subjects and 1 female subject; age range, 28-44 years) and 1 allergic female patient were also included in the analysis. Patient recruitment, informed consent, and sample collection were carried out according to protocols approved by the Institutional Review Board at Stanford University. The clinical trial of influenza vaccination involving these patients is registered at ClinicalTrials.gov (#NCT01827462).

cDNA production, IGH amplification, and high-throughput DNA sequencing

Isolation of PBMCs, RNA extraction, and preparation of isotype-specific IGH libraries were performed by using previously described methods and modified for Illumina instrument sequencing (Illumina, San Diego, Calif).[15,16] Separate barcoded, isotype-specific immunoglobulin libraries for IgM, IgD, IgG, IgA, and IgE were prepared from each sample.[15] Briefly, cDNA was PCR amplified with multiplexed 5′ primer sets for the IGHV FR1 framework regions (FRs) together with 3′ primers specific for exon 1 of the constant region for each isotype. Isotype primers consisted of a gene-specific sequence, a nucleotide sequence barcode, and an additional randomized 4-base sequence, in addition to a partial Illumina instrument primer sequence (see Tables E1-E4 in this article's Online Repository at www.jacionline.org). Separate amplifications were carried out for each isotype to prevent cross-isotype chimeric PCR product formation. After this initial PCR step, a second PCR reaction was performed to complete the Illumina linker sequences at the 5′ and 3′ ends of the immunoglobulin amplicons and to ensure that final PCR reactions were not amplified to saturation. PCR products were gel extracted, pooled, and sequenced with an Illumina MiSeq instrument. Paired end reads were merged by using FLASH,[17] and then identical reads were collapsed to a single representative. Sequences can be accessed through dbGAP (http://www.ncbi.nlm.nih.gov/gap) with accession number phs000666.v1.p1.

Annotation and preprocessing of immunoglobulin sequences

Variable, diversity, and joining gene identity for each immunoglobulin sequence were identified by using IgBLAST[18] after sequence demultiplexing and primer trimming. Isotype and subtype annotation required exact sequence match to non–primer-encoded sequence regions. The framework 3 (FR3)–complementarity determining region 3 (CDR3) boundary was obtained from IgBlast output, whereas the CDR3-FR4 boundary was subsequently identified by using a position weight matrix search trained to identify the WGQG motif and sequence variants at this position. Sequences lacking V segment regions or showing evidence for chimerism derived from PCR amplification were filtered and excluded from further analysis.

Identification of clonally related immunoglobulin sequences

IGH sequences from each participant were grouped into clusters where cluster members possessed (1) the same variable gene segment, excluding allele information; (2) identical CDR3 length; and (3) a minimum of 80% CDR3 nucleotide sequence similarity. The CDR3 similarity threshold was chosen based on comparisons of CDR3 sequence distances within subjects (where true clonally related sequences are present) compared with distances between CDR3 sequences from different subjects (where clonal relationships are not possible but rare similar sequences can be produced independently during receptor rearrangement). Clustering based on nucleotide sequence similarity was accomplished by using the cd-hit-est.[19] For clustered sequences sharing the same randomized 4-mer from the first PCR primer, putative PCR errors derived from the second PCR step of library preparation, as well as sequencing errors, were filtered out by selecting the sequence containing the lowest number of mutations with respect to the germline V and J segments for the rearrangement.

Identification of nearest-neighbor isotype events

Sequences within each cluster were considered to be clonally related members of the same B-cell lineage. To identify sequences within a lineage that share somatic mutations, providing evidence of descent from a shared precursor, we counted the number of shared mutation positions between pairs of sequences of different isotypes. Although by definition all members of a lineage had the same variable gene segment, excluding allele information, differences in allele calling (for variable gene segments) and occasionally diversity and joining gene annotation were observed, likely because of the inherent difficulty in annotating more heavily mutated IGH sequences. Therefore we began by identifying the consensus variable, diversity and joining genes for each lineage, which we defined as the most common V and J gene assignment for the unique sequences within that lineage. Next, mutated positions and the resultant base for each sequence were identified by using