Antibodies have the specificity to differentiate foreign antigens that mimic self antigens, but it remains unclear how such specificity is acquired. In a mouse model, we generated B cells displaying an antibody that cross-reacts with two related protein antigens expressed on self versus foreign cells. B cell anergy was imposed by self antigen but reversed upon challenge with high-density foreign antigen, leading to germinal center recruitment and antibody gene hypermutation. Single-cell analysis detected rapid selection for mutations that decrease self affinity and slower selection for epistatic mutations that specifically increase foreign affinity. Crystal structures revealed that these mutations exploited subtle topological differences to achieve 5000-fold preferential binding to foreign over self epitopes. Resolution of antigenic mimicry drove the optimal affinity maturation trajectory, highlighting the value of retaining self-reactive clones as substrates for protective antibody responses.
and fig. S9). Thus, an antibody that was initially unable to distinguish foreign from self antigen had evolved a 5000-fold differential binding to foreign antigen over self antigen by first mutating away from binding self antigen and subsequently mutating toward binding foreign antigen. SWHEL-derived cells that had lost self-binding but retained foreign binding were also frequent among the IgG1+ memory B cell compartment (fig. S10). Foreign antigen–specific IgG1 serum titers were increased in mice with initially self-reactive SWHEL B cells (fig. S11).

A different, less optimal evolutionary trajectory prevailed when SWHEL B cells were not self-reactive. This trajectory was dominated by acquisition of a CDR2 mutation (Y58F) alone, paired, or in trio with S52T and Y53F (Fig. 3). Y58F alone or with S52T and Y53F increased self-affinity by a factor of four, explaining why this trajectory was not taken by self-reactive SWHEL cells. The Y58F-S52T-Y53F trio increased foreign affinity to $2 \times 10^9$ M$^{-1}$, which was one-third of the affinity obtained with the I29F-S52T-Y53F trio selected through the self-reactive trajectory.

To understand how these three mutations conferred a 5000-fold differential binding to foreign protein over self, we used x-ray crystallography to analyze the structure of HyHEL10(S52F, S52T, Y53F) in complex with DEL (Fig. 4, table S2, and movie SI) compared to that of wild-type HyHEL10 (HyHEL10WT) in complex with HEL (19). I29F resulted in a structural rearrangement of the CDR1 loop to accommodate the larger phenylalanine side chain. Displacement of this loop (arrow 1 in Fig. 4C) opened up additional structural adjustments of CDR2 (arrow 2 in Fig. 4C) (20) and, in particular, repositioned Y53F to interact with the hydrophobic pocket formed on the surface of DEL by the short Ala75 (A75) side chain, which is in contrast to the much longer leucine face of DEL by the short Ala75 (A75) side chain.

The CDR2 backbone adjustments also allowed replacement of the smaller S52 side chain with threonine. Thus, our structural analyses allowed replacement of the smaller S52 side chain with threonine. Thus, our structural analyses in HEL. The CDR2 backbone adjustments also allowed replacement of the smaller S52 side chain with threonine. Thus, our structural analyses in HEL. The CDR2 backbone adjustments also allowed replacement of the smaller S52 side chain with threonine. Thus, our structural analyses...
effect was further confirmed by solving the structure of HyHEL10I29F in complex with DEL (fig. S12).

We next identified anergic B cells in the mHEL3X transgenic (mHEL3Xtg) mice within a polyclonal repertoire that displayed micromolar affinity for the same self antigen and tested whether these B cells too could resolve antigenic mimicry. HEL3X-binding B cells constituted 2.7% of IgD⁺ IgMlo anergic B cells and 0.5% of all splenic B cells (fig. S13A). These were sorted and added at 0.5% frequency to unselected CD45.1⁺ B cells, and the polyclonal mixture was injected together with T cells into mHEL3Xtg Rag1−/− mice immunized with DEL-conjugated SRBCs. In the recipients, 96% of the GC response was derived from the unselected CD45.1⁺ B cells, presumably recognizing mostly SRBC antigens. In contrast, 61% of the DEL-binding GC response was derived from the polyclonal HEL3X-binding anergic CD45.2⁺ B cells (fig. S13B). Only 9.7% of these cells still bound self antigen, whereas 53% bound foreign DEL selectively (fig. S13C). Thus, in a normal repertoire, cells with micromolar affinity for self HEL3X are dominant contributors to the GC response against the self mimic DEL and rapidly lose binding to self.

The findings here extend evidence for antibody redemption in human antibodies (7–9) by showing that mutation away from self-reactivity...
would not be available in individuals treated with antibiotics or raised in a more hygienic environment. The evolution of an antibody along a limited set of mutation trajectories, driven by two selection pressures for higher affinity for one ligand and lower affinity for another, provides an example of deterministic molecular evolution. Our findings provide insights into the GC reaction and the evolution of specificity in antibody-antigen interactions.

REFERENCES AND NOTES


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Author contributions: D.L.B. performed and analyzed mouse experiments; P.S. performed and analyzed binding affinity experiments; D.B.L. performed and analyzed crystallography experiments; J.J. and K.B. generated antibodies and antigens; T.D.C., J.H.R., and J.R.H. developed mHEL3Xtg mice; R.B. devised and developed the mHEL3X × 5Wex system; C.C.G. supervised B cell biology aims; D.C. supervised structural and biophysical aims; D.L.B., P.S., D.B.L., R.B., D.C., and C.C.G. designed and interpreted experiments; D.L.B., D.B.L., P.S., K.P., R.B., D.C., and C.C.G. prepared figures; B.T.P. generated movie SI; and D.L.B. drafted and R.B., D.C., and C.C.G. revised the manuscript.

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Data and materials availability: Coordinates and structure factors have been deposited in the Protein Data Bank with accession codes 5VJO and 5VUQ. All other data needed to evaluate the conclusions in the paper are present in the paper or the supplementary materials.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/360/6385/223/suppl/DC1
Materials and Methods
Fig. S1 to S13
Tables S1 and S2
References (26–35)
Movie SI

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Germinal center antibody mutation trajectories are determined by rapid self/foreign discrimination
Deborah L. Burnett, David B. Langley, Peter Schofield, Jana R. Hermes, Tyani D. Chan, Jennifer Jackson, Katherine Bourne, Joanne H. Reed, Kate Patterson, Benjamin T. Porebski, Robert Brink, Daniel Christ and Christopher C. Goodnow

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**Autoantibody redemption through rapid mutations**
Antibodies distinguish foreign epitopes from closely related self-antigens by poorly understood mechanisms. In mice, Burnett *et al.* found that a proportion of B cells could cross-react with similar foreign and self-antigens (see the Perspective by Kara and Nussenzweig). Challenge with self-antigen resulted in anergy (i.e., a lack of immune response), which was reversed by exposure to high-density foreign antigen. Mutations that decreased self-affinity were rapidly selected for, whereas selection for epistatic mutations that enhanced foreign reactivity took longer. Self-reactivity, rather than being an impediment to immunization, resulted in higher affinities against a foreign immunogen. *Science,* this issue p. 223; see also p. 152