PNAS Plus Significance Statements

Influenza hemagglutinin stem-fragment immunogen elicits broadly neutralizing antibodies and confers heterologous protection

Vamsee V. A. Mallajosyula, Michael Citron, Francesca Ferrara, Xianghan Lu, Cheryl Callahan, Gwendolyn J. Heidecker, Siddhartha P. Sarma, Jessica A. Flynn, Nigel J. Temperton, Xiaoping Liang, and Raghavan Varadarajan

Hemagglutinin (HA), the major influenza virus envelope glycoprotein, is the principal target of neutralizing antibodies. Wide diversity and variation of HA entails annual vaccination, as current vaccines typically fail to elicit/boost cross-reactive, broadly neutralizing antibodies (bnAbs). Although several bnAbs bind at the conserved stem of HA making it an attractive universal vaccine candidate, the metastable conformation of this domain imposes challenges in designing a stable, independently folding HA stem immunogen. We rationally designed a stem-fragment immunogen (pp. E2514–E2523), mimicking the native HA stem that binds conformation-specific bnAbs with high affinity. The immunogen elicited bnAbs and conferred robust protection against lethal, heterologous virus challenge in vivo. Additionally, soluble bacterial expression of such a thermotolerant, disulfide-free immunogen allows for rapid scale-up during pandemic outbreak.

Cofactor-dependent conformational heterogeneity of GAD65 and its role in autoimmunity and neurotransmitter homeostasis

Itamar Kass, David E. Hoke, Mauricio G. S. Costa, Cyril F. Reboul, Benjamin T. Porebski, Nathan P. Cowieson, Hervé Leh, Eugenia Pennacchietti, Julia McCoey, Oded Kleifeld, Carla Borri Voltattorni, David Langley, Brendan Roome, Ian R. Mackay, Daniel Christ, David Perahia, Malcolm Buckle, Alessandro Paiardini, Daniela De Biase, and Ashley M. Buckle

Autoimmune type 1 diabetes is characterized by the formation of self-reactive antibodies. A prevalent human autoantigen is glutamate decarboxylase (GAD)65, a highly predictive marker that can precede the emergence of disease by up to several years. Intriguingly, the closely related isoform GAD67 is not immunogenic. What are the determinants of the unique self-reactivity of GAD65 vs. GAD67? We show that, unlike GAD67, GAD65 is highly flexible and exists in multiple structural forms. We show (pp. E2524–E2529) that self-antibodies bind differentially to these GAD65 forms. These properties may be an undesirable consequence of conformational flexibility necessary for enzyme function. Our findings, thus, provide insights into how structural flexibility governs protein immunogenicity in autoimmune diabetes and have implications for therapeutic antibody and vaccine design.

Taxodione and arenarone inhibit farnesyl diphosphate synthase by binding to the isopentenyl diphosphate site

Yi-Liang Liu, Steffen Lindert, Wei Zhu, Ke Wang, J. Andrew McCammon, and Eric Oldfield

There is an ever-present need for new drugs because of drug resistance. An enzyme called "farnesyl diphosphate synthase" (FPPS) is one important drug target, and drugs called "bisphosphonates" that inhibit this enzyme are of interest both as cancer therapeutics and as antibacterial and antiparasitic drug leads. However, they bind avidly to bone and so are ineffective against most tumors and most infectious organisms. Here (pp. E2530–E2539), we report the discovery of compounds that lack a bone-binding feature that target FPPS in a unique way, as observed at the atomic level. They also can bind to other protein targets, providing a potentially important approach, multitarget inhibition, that is expected to increase efficacy and decrease the likelihood that resistance will develop.

Antigen expression level threshold tunes the fate of CD8 T cells during primary hepatic immune responses

Szun Szun Tay, Yik Chun Wong, David M. McDonald, Nicole A. W. Wood, Ben Roediger, Frederic Sierro, Claire Mcguffog, Ian E. Alexander, G. Alex Bishop, Jennifer R. Gamble, Wolfgang Weninger, Geoffrey W. McCaughan, Patrick Bertolino, and David G. Bowen

The liver possesses unique immunological properties, with the capability of inducing tolerance upon transplantation, yet is also the target of immune-mediated damage in chronic viral hepatitis. To investigate the basis of these dichotomous outcomes, we manipulated several determinants capable of influencing outcomes of hepatic–immune interactions. Our findings (pp. E2540–E2549) reveal that a threshold of antigen expression within the liver is the dominant factor determining the fate of CD8 T cells recognizing intrahepatic antigen, irrespective of their affinity for antigen or the site of initial antigen encounter, with high-level antigen expression leading to exhaustion of T cell function. To our knowledge, for the first time, this study provides a unified model explaining the divergent consequences of hepatic–immune interactions.

Distinct phases in the positive selection of CD8$^+$ T cells distinguished by intrathymic migration and T-cell receptor signaling patterns

Jenny O. Ross, Heather J. Melichar, Byron B. Au-Yeung, Paul Herzmark, Arthur Weiss, and Ellen A. Robey

Developing T cells are positively selected in the thymus to ensure that their antigen receptors can interact with self-MHC. For CD8 T cells, this process takes days to complete, yet the steps involved are poorly understood. We followed a synchronized wave of cells undergoing positive selection within three-dimensional thymic tissue. Surprisingly, migration from the cortex to the medulla occurred before CD4 down-regulation and while thymocytes still required TCR signaling for efficient positive selection. There was a gradual change in the pattern of calcium signaling over time, with an upward shift in basal intracellular calcium correlating with increased speed and brief signaling events. Our data (pp. E2550–E2558) have interesting implications for how positive and negative selection shape the mature CD8 T-cell repertoire.

Nod/Ripk2 signaling in dendritic cells activates IL-17A–secreting innate lymphoid cells and drives colitis in *T-bet*$^{-/-}$.*Rag2*$^{-/-}$ (TRUC) mice

Joerg Ermann, Tracy Staton, Jonathan N. Glickman, Rene de Waal Malefyt, and Laurie H. Glimcher

Disease mechanisms in inflammatory bowel disease (IBD) are incompletely understood. In this study, we analyzed the role of IL-17A–secreting innate lymphoid cells (ILCs) in a mouse model of microbiota-driven innate immune-mediated colitis. We report (pp. E2559–E2566) that the pathogenic IL-17A response in ILCs is controlled indirectly by microbial stimulation of dendritic cells (DCs) via the nucleotide-binding oligomerization domain containing (Nod)/receptor-interacting serine-threonine kinase 2 (Ripk2) signaling pathway and requires the cytokines IL-23 and IL-1. Insight into the complex interactions between various immune cells as demonstrated here for DCs and ILCs is a prerequisite for the development of more efficacious therapies in IBD.

Redemption of autoantibodies on anergic B cells by variable-region glycosylation and mutation away from self-reactivity

Zahra Sabouri, Peter Schofield, Keisuke Horikawa, Emily Spierings, David Kipling, Katrina L. Randall, David Langley, Brendan Roome, Rodrigo Vazquez-Lombardi, Romain Rouet, Jana Hermes, Tyani D. Chan, Robert Brink, Deborah K. Dunn-Walters, Daniel Christ, and Christopher C. Goodnow

Antibodies are selected to bind microbial but not self-antigens, because binding to self would compete with binding microbes, shorten antibody half-life, and cause autoimmunity. Self-tolerance is actively acquired in part by discarding self-binding antibodies before the body is exposed to a microbe or vaccine. The experiments here (pp. E2567–E2575) provide evidence of an opposite mechanism, allowing antibodies that initially bind both foreign and self-antigens to acquire self/non-self discrimination during the course of an immune response through somatic hypermutation away from self-reactivity. In addition to selection for lower-affinity binding to self, antibody variants were selected with fewer binding sites available to bind self-antigen because most were occupied by N-linked carbohydrate, possibly explaining the frequent occurrence of N-linked glycosylation of antibody variable domains.

Correcting direct effects of ethanol on translation and transcription machinery confers ethanol tolerance in bacteria

Rembrandt J. F. Haft, David H. Keating, Tyler Schwaegler, Michael S. Schwalbach, Jeffrey Vinokur, Mary Tremaine, Jason M. Peters, Matthew V. Kotlajich, Edward L. Pohlmann, Irene M. Ong, Jeffrey A. Grass, Patricia J. Kiley, and Robert Landick

Microbially produced aliphatic alcohols are important biocommodities but exert toxic effects on cells. Understanding the mechanisms by which these alcohols inhibit microbial growth and generate resistant microbes will provide insight into microbial physiology and improve prospects for microbial biotechnology and biofuel production. We find (pp. E2576–E2585) that *Escherichia coli* ribosomes and RNA polymerase are mechanistically affected by ethanol, identifying the ribosome decoding center as a likely target of ethanol-mediated conformational disruption and showing that ethanol inhibits transcript elongation via direct effects on RNA polymerase. Our findings provide conceptual frameworks for the study of ethanol toxicity in microbes and for the engineering of ethanol tolerance that may be extensible to other microbes and to other short-chain alcohols.

DNA looping-dependent autorepression of *LEE1* P1 promoters by Ler in enteropathogenic *Escherichia coli* (EPEC)

Abhayprasad Bhat, Minsang Shin, Jae-Ho Jeong, Hyun-Ju Kim, Hyung-Ju Lim, Joon Haeng Rhee, Soon-Young Paik, Kunio Takeyasu, Toru Tobe, Hilo Yen, Gwangrog Lee, and Hyon E. Choy

Ler [locus of *e*nterocyte *e*ffacement (LEE)-encoded regulator], encoded by the first gene of the LEE1 operon in enteropathogenic *Escherichia coli* (EPEC), represses its own transcription driven by two promoters separated by 10 bp. We found (pp. E2586–E2595) that Ler does this repression through a DNA loop of 16 helical turns, in which RNA polymerase is trapped as open promoter complex, although this complex should be most readily transformed into productive initiation complex.

Probing the paramyxovirus fusion (F) protein-refolding event from pre- to postfusion by oxidative footprinting

Taylor A. Poor, Lisa M. Jones, Amika Sood, George P. Leser, Manolo D. Plasencia, Don L. Rempel, Theodore S. Jardetzky, Robert J. Woods, Michael L. Gross, and Robert A. Lamb

The activities of the fusion proteins that mediate virus–cell fusion are an absolute requirement for virus entry and infectivity of enveloped viruses such as HIV, influenza virus, measles virus, and respiratory syncytia virus, among others. Viral fusion proteins are translated initially in a metastable prefusion state and, upon triggering, undergo an extensive and irreversible refolding process. Membrane fusion is coupled to the energy released by the fusion proteins adopting a stable, low-energy postfusion state. Here (pp. E2596–E2605) we use oxidative footprinting of the parainfluenza virus 5 fusion protein to reveal new details of this critical event in the viral lifecycle. A greater understanding of the dynamic nature of these metastable proteins may reveal novel opportunities for the development of targeted therapeutics.

Immersive audiomotor game play enhances neural and perceptual salience of weak signals in noise

Jonathon P. Whitton, Kenneth E. Hancock, and Daniel B. Polley

All sensory systems face two fundamental limitations: (*i*) segregating partially overlapping sensory inputs into separate perceptual objects and (*ii*) raising sensory events that are either weak or noisy to perceptual awareness. The ability of sensory systems to extract information from weak signals in noisy backgrounds can improve with practice, but learning does not typically generalize to untrained stimuli. By training humans and mice with an audio game inspired by sensory foraging behavior, we show (pp. E2606–E2615) that learning to discriminate simple, easily controlled sounds can generalize to improved neural and perceptual processing of "real-world" complex sounds, including speech in noise. These findings suggest new therapeutic options for clinical populations that receive little benefit from conventional sensory rehabilitation strategies.

Evolutionary developmental transcriptomics reveals a gene network module regulating interspecific diversity in plant leaf shape

Yasunori Ichihashi, José Antonio Aguilar-Martínez, Moran Farhi, Daniel H. Chitwood, Ravi Kumar, Lee V. Millon, Jie Peng, Julin N. Maloof, and Neelima R. Sinha

Ever since Darwin's pioneering research, a major challenge in biology has been to understand the genetic basis of morphological evolution. Utilizing the natural variation in leaf morphology between tomato and two related wild species, we identified a gene network module that leads to a dynamic rewiring of interactions in the whole leaf developmental gene regulatory network. Our work (pp. E2616–E2621) experimentally validates the hypothesis that peripheral regions of network, rather than network hubs, are more likely to contribute to evolutionary innovations. Our data also suggest that, likely due to their bottleneck location in the network, the regulation in KNOX homeobox genes was repeatedly manipulated to generate natural variation in leaf shape.

Antibody repertoire deep sequencing reveals antigen-independent selection in maturing B cells

Joseph Kaplinsky, Anthony Li, Amy Sun, Maryaline Coffre, Sergei B. Koralov, and Ramy Arnaout

Antibodies play essential roles in vaccination, infection, autoimmunity, aging, and cancer. A key question is how the antibody repertoire achieves its remarkable diversity. Part of the answer is that B cells, which express antibodies on their surface, are selected for survival based on the specific antigens that their antibodies bind, with antigen specificity determined by the protein sequence of antibodies' antigen-binding regions. Unexpectedly, we find (pp. E2622–E2629) that B cells are also selected based on whether their antibodies have a loose or tight "elbow joint," independent of the sequence of their antigen-binding regions. This discovery, enabled by sequencing technology and mathematics, adds a surprising new dimension to our understanding of antibody repertoires, and might one day help us shape them ourselves.